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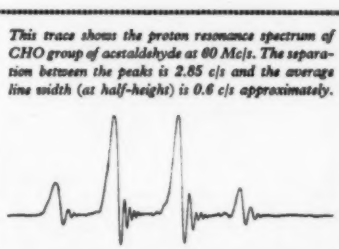

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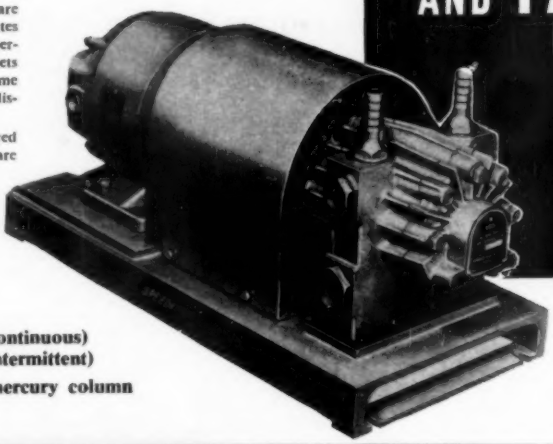

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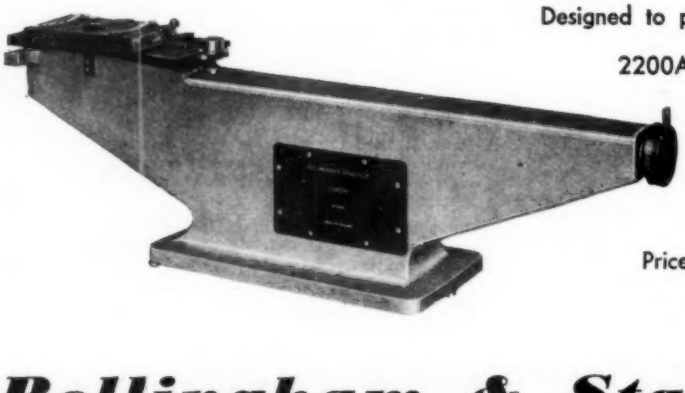

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LIBRARIES AND LIBRARY SERVICE IN BRITAIN

ALTHOUGH the question of scientific and technical libraries was virtually ignored in the recent annual report of the Advisory Council for Scientific Policy, a full session of the Home Universities Conference in December 1959 was devoted to a discussion on university libraries. One of the participants in that discussion, Mr. B. S. Page, was this year's president of the Library Association, and in his annual address to the Association at Scarborough in September—when Dr. D. J. Urquhart outlined the ideas and motives behind the development of the National Lending Library for Science and Technology—he stressed once again the human aspect of librarianship and the demands made on the librarian for scholarship, as well as for human understanding and imaginative insight into the needs of the users of his library. That was, in fact, one of the dominant notes struck in the discussion last winter at the Home Universities Conference*, which was particularly concerned that in the planning and organization of a university library the point of view of the reader, and indeed his weaknesses, should not be ignored. Here, perhaps, as nowhere else, the value of 'browsing' is greatest and special efforts are justified to ensure the maximum access. Prof. J. M. R. Cormack quoted with approval Justin Winsor's principle that books should be used to the largest extent possible and with the least trouble, and observed that it was often the chance find on the shelves that was most useful.

It should not be supposed that either the Advisory Council for Scientific Policy or the University Grants Committee is likely to be altogether unmindful of the human aspects of an adequate library system, whether in the universities or elsewhere, or to forget that however adequate the provision made, its effective use will depend to a great extent on the men and women who operate the service. Men and women of the high calibre and enthusiasm desired will not be forthcoming unless they are given both the tools for the job and accorded the status and respect that go with the scholarship and administrative responsibility involved. These professional demands are growing, and it is important that when the physical needs of university libraries and of a national library service, of which it should be remembered university libraries form an indispensable part —they supplied 28,611 books to the National Central Library in 1959–60 as against the 12,793 they received—are steadily growing, this aspect is not overlooked. Indeed, the co-operation which is essential if an adequate national system is to be developed itself makes considerable demands on the personality and administrative ability of the librarian. It is not simply a matter of techniques and mechanical devices.

The latter, however, is the only aspect to which the Advisory Council on Scientific Policy refers in the section on library work in its current report. Recognizing the immensity of the problem created by the enormous growth of the output of scientific literature in all countries, the Advisory Council suggests that this problem will continue increasingly to impede the utilization of scientific and technical information, and so progress, unless it is recognized as meriting research in its own right; techniques must be devised for recording and presenting scientific information in a form in which its significance can be most readily appreciated by those who have to use it. The Council considers that serious research into the whole problem of collation and dissemination of scientific information should be undertaken forthwith, possibly under the auspices of the new National Science Lending and Reference Libraries and in association with international efforts in this sphere.

The Advisory Council does not mention the Association of Special Libraries and Information Bureaux, of which the terms of current grant from the Department of Scientific and Industrial Research require it to undertake research into the field of documentation; but at least its remarks show that the Advisory Council is aware both of the magnitude of the problem and of the need for co-operation up to the international level. The enormous increase in the output of scientific literature is, however, not reflected in the returns for library expenditure given by the University Grants Committee. While annual expenditure on libraries had risen from £156,000 in 1925–26 to £255,000 in 1938–39, £988,000 in 1951–52 and £1,979,945 in 1959–60, the doubling of expenditure during the past decade has been spread fairly evenly over the different items. Expenditure on salaries and wages rose from £527,238 to £1,070,466; on books from £202,567 to £434,254; and on periodicals from £118,702 to £231,458. The percentage which library expenditure represents to the total expenditure remains at 3·8.

It is, however, the figures for the expenditure of individual libraries that are most illuminating. Apart from the University of London, with expenditure on books of £91,817 (£12,301 was for the Central Library and £13,526 for University College), the only university libraries in Britain spending more than £20,000 on books in 1959–60 are those which are entitled to receive on demand a copy of every published book in the United Kingdom, namely, Oxford (£62,088) and Cambridge (£28,734). Indeed, outside Scotland, where Edinburgh, Glasgow and St. Andrews spent more than £19,000, £18,000 and £16,000, respectively, on books, only Manchester, Leeds, Birmingham, Durham, Liverpool and Sheffield spent more than

* Association of Universities of the British Commonwealth. Conference of the Universities of the United Kingdom—Report of Proceedings. Pp. 104. (London: Association of Universities of the British Commonwealth, 1960.) 5s.

£10,000. The picture with expenditure on periodicals is similar. A quarter of London's expenditure of £63,173 was for the Central Library and that of University College. The University of Cambridge spent £17,400 on periodicals and the University of Oxford £15,461; only three other universities spent more than £10,000—Glasgow, Manchester and Birmingham—though Edinburgh spent only just short of this figure. Furthermore, the proportion which library expenditure bears to the total expenditure also varies widely, especially among the English universities—from 8·3 per cent for Oxford and 7·2 for the Durham Colleges of the University of Durham to 2·0 for King's College of the same University and 0·9 for the Manchester College of Science and Technology; and within the University of London, which averages 3·5, from 36·5 for special institutions like the Courtauld Institute of Art, 7·7 for the London School of Economics and also for the Central Library, to 0·9 for the Imperial College of Science and Technology—a figure attributed to the access enjoyed to the Science Library.

How far these figures are really comparable cannot be judged from the tables supplied by the University Grants Committee, but clearly some account must be taken both of the existing resources of a university and its size. Apart from the fact that some figures like that for the Manchester College of Science and Technology are clearly abnormal—in this instance through inclusion under miscellaneous university expenditure of a surprisingly high figure for interest on overdrafts, etc.—it is common knowledge that expenditure on books for departmental libraries is sometimes included under departmental expenditure and sometimes under library expenditure. It is noteworthy that in the new institutions such as the University College of North Staffordshire (5·7), University of Hull (5·4), University of Exeter (5·0), University of Leicester (5·3) and University of Southampton (4·8) the percentage of library expenditure to total expenditure is above the average. Mr. K. W. Humphreys, librarian of the University of Birmingham, in opening the discussion at the Home Universities Conference, pointed out that already expenditure on libraries exceeds the total Government grant to universities a generation ago. Even at the existing rate of growth, £16 million might well be required for libraries by the late 'eighties, and if any attempt is made to overtake the rapid increase in student population after the mid 'seventies, libraries should absorb an increasing proportion of the general university grant—possibly 10–15 per cent by the end of the century.

So long ago as 1921, the University Grants Committee expressed the view that the character and efficiency of a university might be gauged by its treatment of its central organ, the library. "We regard the fullest provision for library maintenance as the primary and most vital need in the equipment of a university. An adequate library is not only the basis of all teaching and study, it is the essential condition of research, without which additions cannot be made to the sum of human knowledge." In its report on "University Development" two years ago, the Committee recognized that a large part of university expenditure for research of all kinds is expenditure on libraries, and that although between 1951–52 and 1956–57 expenditure on libraries had increased by 64 per cent, this was largely due to increased costs, and the real increase was less than 10 per cent.

Mr. Humphreys recognized that the new National Lending Library for Science and Technology should relieve some of the pressure on the universities inter-lending scheme and provide copies of out-of-the-way periodicals so that science and technology can be better served; but even this passing reference indicates that the universities cannot stand right outside the library services of Great Britain, and Dr. Urquhart, in speaking at Scarborough, reiterated his view that demands for inter-library lending are likely to grow. Mr. Humphreys believes that while the undergraduate population has increased, their book buying has declined; this has been challenged, but even if the Anderson Committee's recommendation that an allowance for books should be included in all maintenance grants and the figure made public has its full effect, the university library is unlikely to benefit substantially. As Mr. Humphreys himself pointed out, the best hope of relieving pressure lies in co-operative action, in the adoption where possible of new techniques and in skilled administration—the same factors that alone can ensure the most efficient use of whatever resources may be available.

Some of these problems were indicated in an illuminating address by Prof. J. M. R. Cormack, who, approaching the problem from the reader's point of view, displayed very clearly not merely some of the opportunities for improvement but also the pitfalls that attend the unwary adoption of new gadgets. It was left, however, for Mr. Humphreys, in replying to the discussion, to refer to some of the implications of the suggestions which had been made in regard to such matters as inter-university co-operation and longer hours of opening. Questions of staff and of finance as well as education may be involved, running well outside the university library itself.

This discussion at last year's Home Universities Conference shows clearly that the universities themselves are well aware of the importance of the university library and the vital contribution it makes to the efficiency with which a university performs its functions of teaching and research. Moreover, those in charge of the university libraries are obviously alive alike to the varied needs of the users of those libraries, whether they be undergraduates or members of the university's staff; and they are no less aware of the important place which the university library holds in the library system of Great Britain as a whole. The way in which criticism advanced in the course of the discussion was received is no less encouraging; weaknesses in the university libraries should not be charged primarily against those

responsible for their administration, but against those responsible for failure to provide the resources commensurate with the growing demands which the universities and the community make on the university libraries.

How far those weaknesses are the responsibility of the University Grants Committee itself or of individual universities can scarcely be judged from the published evidence. All that can be said is that, judged in terms of total expenditure on libraries, expenditure on books, periodicals or staff, or the proportion which library expenditure bears to university expenditure as a whole—or on the expenditure per head of staff and student as noted by Dr. W. S. Mitchell in the discussion—the expansion of expenditure on libraries falls a long way short of that required to meet the ideal formulated by the University Grants Committee more than a generation ago. What is clearly needed is much firmer support from the University Grants Committee and also a wider public understanding generally.

Mr. B. S. Page, in his presidential address to the Library Association, manifestly sought to enlist such support and understanding from the Library Association, and there should be other opportunities in the present Parliamentary session which might be used. In spite of the fact that the Roberts Committee on the structure of the public library service was singularly indifferent to the part played by the universities, that omission should be rectified whenever the Minister of Education advances his proposals for action on the report, though that is unlikely to be in the present session—a fact which itself indicates how little importance the Government attaches to the provision of an adequate library service. It is, however, on the Advisory Council for Scientific Policy and on the Minister for Science that the major responsibility rests. Dr. Urquhart's address to the Library Association shows that he is aware of the inter-relations between the university libraries and the new National Lending Library for Science and Technology, and that he is indeed anxious to foster co-operation and on the proper basis—of ascertained fact. Dr. Urquhart, however, is only responsible for the lending side, and the National Reference Library for Science and Technology also comes into the picture.

It is only as the Minister and the Advisory Council ensure that that project is vigorously and adequately developed, and due account taken not only of the resources and functions of the university libraries and of those of the colleges of technology, but also of the technical libraries provided by at least the major public libraries, that we can hope to see, inside and outside the universities, a library service adequate to meet the needs of the whole community in this age of rapid technological change. It has to be admitted that so far the Minister and the Advisory Council have shown little sign that they are aware of the immensity of their responsibility in this field, or of its far-reaching implications. One remark at least in a discussion at the Library Association's Conference at Scarborough should bring that responsibility home. Commenting on Mr. M. C. Pottinger's address on "Libraries in the Soviet Union", Mr. L. L. Ardern, of the Manchester College of Science and Technology, remarked that the need for a British Scientific Information Centre had been very fairly illustrated, and Mr. Pottinger fully concurred. When all recognition has been made of Dr. Urquhart's readiness to see the new National Lending Library for Science and Technology functioning as part of a truly national system, it is difficult to reconcile its location in Lincolnshire, or the tardiness with which the corresponding reference library project is developing, with any sense at the centre of the inter-relations inherent in a national system, or of the savings which could have been achieved by real planning in relation to the resources of the whole country. Little account seems to be taken of the contributions which the university libraries and those of the learned societies and professional institutions, as well as the National Central Library and the public libraries themselves, could make. In few other fields is piecemeal development more costly in terms of finance and of goodwill.

DARWIN PARTIALLY APPRAISED

Darwin's Place in History

By Prof. C. D. Darlington. Pp. ix+101. (Oxford: Basil Blackwell, 1959.) 9*s*. 6*d*.

PROF. DARLINGTON is perhaps unfortunate in the fact that his essay, dated February, was not published until the late December of 1959, the year when a spate of publications celebrating the centenary of the "Origin" almost overwhelmed the reader. His thesis does not therefore strike home with the originality which its initial composition presumably required. Charles Darwin is shown in this publication to have used the work of precursors in the field of transmutation without sufficient acknowledgment; to have equivocated over the problem of the origin and transmission of variation in Nature; to have "failed to recognize or to relate his own ideas . . . with those of others working in the same field"; and to have used to help achieve his admitted "success in mass conversion" to the evolutionary hypothesis "a flexible strategy which is not to be reconciled with even average intellectual integrity". Darwin is given credit for independence of thought, but this he owed not to any personal quality but to the fact that "by independence of economic position, he had a unique advantage over all his contemporaries".

This remarkable and original view is presented with a pretty wit and a challenging directness. Nobody reading this pamphlet could fail to be stimulated by it. The author is master of the telling phrase, for example, when he refers to Darwin "assembling his forces with a prudent strategy without which he would never have won his battle"; his remark that historians of science "often believe what the discoverer writes about his own discoveries, which, as we see, is not a wise thing to do"; and his comment on Samuel Butler's last anti-Darwin

book "its title *Luck or Cunning* referred not to the causes of Darwin's success but to Butler's views on evolution".

Darlington makes a clear distinction between 'hard' and 'soft' heredity. Hard heredity implies selection after variation and soft heredity direction before variation. In an appendix he supplies an anthology of great value for the student from the writings of protagonists of both views. It is fortunate that the original language is sometimes given so the reader may reach his own conclusion concerning the propriety of translating "genitalia corpora" in Lucretius as "genetic bodies".

Authors are frequently presented in language, admittedly colourful, which carries undesirable overtones of sense acquired in more recent usage. Lawrence, for example, is credited with the use of 'mutation' though he actually wrote "native or congenital varieties". It is somewhat disconcerting to find that Prof. Darlington seems unaware that until the 1850's the word evolution was used in an embryological as well as in its later (post-Darwin) transmutation sense, for he comments (p. 35) that T. H. Huxley's (1878) essay on the history of evolutionary theory was "curiously combined with a history of embryology" although one of his own quotations from Spencer (p. 83) speaks of the physiologist "who knows that every individual being is so evolved" from "their earliest condition" when "the germs of all plants and animals whatever are so similar". Nor is it correct to imply that Darwin did not begin to write the "Origin" until he received Wallace's essay for publication, or that Darwin considered all differences between parents blended in their offspring. On this latter point, Darwin in 1837 wrote opposite Lyell's phrase, "Some acquired peculiarities of form, structure, and instinct are transmissible", the comment "with no tendency to go back" and "the very character of species is character being heredity", an unexceptionable statement of 'hard' heredity[1].

Erasmus Darwin as "the great precursor" is well presented in this pamphlet, though it might be made clear to the reader, since Prof. Darlington places so much weight on Charles's reading of his grandfather's works while at Edinburgh, that he arrived there a boy of sixteen and stayed for less than two years.

The claims of Lawrence as a precursor and advocate of 'hard' heredity are seriously advanced by Darlington. Lawrence's lectures, published in 1819, argue at length, and somewhat repetitiously, to demonstrate "that the human species, therefore, like that of the cow, sheep, horse, and pig, and others, is single, and that all the differences, which it exhibits, are to be regarded merely as varieties". There is no evidence that Darwin read this work before 1847, when his ideas were already fully developed. His copy, at Down House, of these lectures is the 1822 Benbow edition. The notes on the back flyleaf include "Zool. acceptation of Species etc. 225–232", but no comment in written on these text pages. What is useful in Lawrence and Prichard was most likely transmitted to Darwin through Lyell's works, of which Darwin wrote to Horner (August 29, 1844): "I always feel as if my books come half out of Lyell's brains & think I never acknowledge this sufficiently . . .". It seems Darwin did not follow up all Lyell's references until a comparatively late date. He, perhaps justifiably, felt he could trust Lyell.

I have said enough about Prof. Darlington's approach to the writing of history for the reader to realize the need for caution in accepting such statements (p. 52) as: "The cellular basis of reproduction and heredity . . . had been revealed in the previous (to 1864) thirty years". The first published description of the fusion of the fertilizing sperm with the egg and the subsequent behaviour of the male and female pronuclei was given in 1874 by Auerbach from his study of nematode worms. Weismann first published on heredity in 1883, one year after Darwin's death.

This sketch of Darwin's place in history seems to have begun as a brilliant impression; unfortunately, the selective heightening of some strokes by the author has caused the sketch at times to become a caricature. It seems as if the author was using opera glasses the wrong way round, so shrunken and inconspicuous is Darwin's image. The reader often has cause to wonder why so much trouble should be taken to demolish such a minor figure. If, in the pursuit of an answer, he turns yet again to Darwin's writings, good will result from this publication.

SYDNEY SMITH

[1] Smith, S., *Adv. Sci.*, **64**, 391 (1960).

MEASURING MOLECULES

Molecular Structure

The Physical Approach. By J. C. D. Brand and J. C. Speakman. Pp. viii+300. (London: Edward Arnold (Publishers), Ltd., 1960.) 30*s*. net.

BRAND and Speakman have set out to give an account of the physical methods of elucidating molecule structure "written at a level suited to the honours student, or to the research worker in some other branch of Chemistry". Students in Glasgow must be strong men, however, if the mathematical level of this volume causes them no trouble. To meet the irreducible representations of a group by p. 26 seems over-demanding for a chemistry student. Like the authors, I certainly wish that all chemistry students had this sort of understanding of the methods of studying molecular structure; but to start with "Brand and Speakman" would be to push most of them in at the deep end. It is difficult to avoid a comparison with P. J. Wheatley's recent book, "The Determination of Molecular Structure", which covers similar ground. Wheatley kept the mathematics to a much simpler level and provided rather more examples of chemical interest, with numerous illustrations of the shapes of molecules. For an introduction to the subject, students would therefore be well advised to start with Wheatley. The abler ones could then pass to Brand and Speakman, who will take them much closer to the mathematical techniques which are the necessary stock-in-trade of the research investigator. By comparing and contrasting the two books, which often complement each other, good students could reach an excellent appreciation of the subject.

The reader who is not troubled by the mathematics will find Brand and Speakman's account in general admirable. The headings of the various chapters on physical methods will serve to show the range they cover: "The Rotation of Molecules", "Nuclear Resonance Spectra", "The Vibration of Molecules", "Anisotropic Polarisability and the Raman Effect", "Crystal Structure Analysis", and "Electron Diffraction". The stage for these is set by two chapters on symmetry and elementary wave mechanics. The latter, which

accidentally, has a clear and sound postulatory approach to the subject, provides the quantum mechanical results needed for the spectroscopic chapters. Almost always the book is thorough and clear. The chapter on nuclear resonance spectra, for example, covers nuclear magnetic resonance in solids and liquids, pure nuclear quadrupole resonance and nuclear quadrupole coupling in pure rotation spectra. A separate chapter on the electron diffraction of gases is especially welcome, as the capabilities of this powerful method of structure investigation have been far too little appreciated in Britain. On the other hand, the discussion of electron and neutron diffraction by crystals, which is part of the chapter on crystal structure analysis, is perhaps too brief. Otherwise this chapter gives a very readable account of the methods and of the range of the results of modern X-ray crystallography.

The final chapter of appraisement and conclusions draws the various threads together and makes useful comparisons of the results of the different methods. The last sentence, however, is very curious. As the conclusion of a contrast of diffraction and spectroscopic techniques, it reads: "Because the spectroscopic methods look towards the wave function, they constitute the most fundamental approach to molecular structure". The implication that diffraction techniques are unrelated to the wave functions is, of course, quite false: a glance at James's standard treatise "The Optical Principles of the Diffraction of X-rays" will show the closeness of their connexion. This extraordinary remark is perhaps the result of a certain unbalance in the authors' treatments of spectroscopic and diffraction methods. They give the wave mechanical fundamentals of spectroscopic theory in some detail, but do not even sketch the corresponding parts of diffraction theory: the key relation $\rho = e\psi^*\psi$ is unmentioned.

Nevertheless, "Brand and Speakman" is excellent value. Its price should induce many students to make the mathematical efforts necessary to achieve a deep understanding of the subject.

Minor flaws noted include misprints in the character table for the point group T_d, where the translations and rotations are assigned to the wrong representations. Fig. 3.5.1 omits any scale for x.

D. W. J CRUICKSHANK

PROGRESS IN NUCLEAR PHYSICS

Progress in Nuclear Physics
Vol. 7. Edited by Prof. O. R. Frisch. (Progress Series.) Pp. vii+326. (London and New York: Pergamon Press, 1959.) 84*s*. net.

THE volume under review covers the following topics: "The Bubble Chamber" (D. V. Bugg), "Resonance Fluorescence in Nuclei" (F. R. Metzger), "Spallation" (B. G. Harvey), "Optical Model for Nuclear Scattering" (A. E. Glassgold), "Measurement of Helicity" (L. Grodzins), and "Weak Interactions" (J. J. Sakurai).

Though an article on the bubble chamber was published in the same series two years ago, a progress report seems indicated in view of the technical advances made and the realization that here we have indeed one of the key instruments for future high-energy research. The theory of chamber operation, the practical considerations associated with various liquids, some design features of operating chambers and much of the auxiliary equipment such as magnet characteristics, illumination, photographs and track-measuring devices are included in the account. Since the bubble chamber produces prolific amounts of detailed information, great efforts will be required to develop methods to extract the information contained in the photographs at the same rate as the chamber records events; here the author might have been more generous in attacking such a formidable task, particularly in so far as the general principles are concerned. It would have been useful to provide more information of the financial side of a bubble chamber project.

Dr. Glassgold's review is most ably done; unfortunately it does not include in the analysis the very important results of polarization effects obtained by Dr. L. Rosen at Los Alamos. This and some other aspects might well be a good topic for a future contribution to this series. Again, the article on resonance radiation, though very well done, is just a trifle stale as it misses the impact of the Mössbauer method in the field. The well-written but extensive review on spallation convinces the reader that this field seems a bit dull and is by now probably exhausted. Choice of the highlights of the volume may be a matter of taste, but I feel few will dissent if this distinction is confined to the two articles, "Measurement of Helicity" (75 pages) and "Weak Interactions" (71 pages). Clarity, completeness (including a discussion on unsolved problems in Dr. Sakurai's article) and lively language all contribute to the joy of reading these surveys.

The literature references seem to include the papers up to 1958, but not 1959; the volume is therefore slightly out of date.

E. BRETSCHER

GAS CHROMATOGRAPHY

Gas Chromatography
By A. I. M. Keulemans. Second edition. Edited by C. G. Verver. Pp. xxi+234. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1959.) 60*s*. net.

THE first edition of Keulemans's "Gas Chromatography", published in 1957, was a landmark in the subject. Prof. Keulemans had played a leading part in the development of both the experimental and theoretical parts of the technique, and was one of the first to direct attention to the importance of operating columns under optimum conditions. The theoretical sections were therefore particularly good and the practical sections gained from a wide practical experience. Since 1957, gas chromatography has developed tremendously, the number of papers having risen from a few hundred to more than three thousand, and in 1958, when the revision of the first edition was presumably being carried out, two highly significant advances occurred. These were the invention of several highly sensitive detectors based on the ionization of the eluted vapours, and of capillary columns. It is now clear that these advances have so altered our outlook on gas chromatography, its aims and potentialities, that any text written before 1958 would require extensive revision to bring it up to date. The revision which has taken place between the first and second editions of "Gas Chromatography" now appears inadequate, although, to be fair, this

was probably not obvious at the time. Only four pages are devoted to the ionization detectors and only four to capillary columns. The Golay equation is not mentioned and there is little in the way of practical information on the operation of capillary columns. There is only the briefest of sections on temperature-programmed gas chromatography and nothing on preparative gas chromatography. Recent work on the improvements in efficiency of packed columns is treated rather sketchily and the katharometer theory remains unaltered in spite of the realization that forced convection is important in determining both the sensitivity and the stability of katharometers. Most of the theoretical sections are still first class, but even here one feels that an improvement could have been effected by more concise presentation now that the initial barriers to the mathematics have been broken down.

My chief impression of the second edition is therefore that modern developments, major and minor, have been given too little space. I am disappointed that so eminent an authority should have failed to make the same mark with his second edition as with his first.

Nevertheless, Keulemans' book is still the best on the subject and can be warmly recommended to those who do not possess the first edition. Those who do must still await an up-to-date account of recent work.

JOHN H. KNOX

LIPIDS AND LIPOPROTEINS

Lipide Chemistry

By Dr. Donald J. Hanahan, Dr. Frank R. N. Gurd and Prof. Irving Zabin. Pp. ix+330. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1960.) 80*s*. net.

AFTER a short introduction, the main chapters have the following sequence: isolation and purification, phosphoglycerides, phosphoinositides, sphingolipids, minor phospholipids, simple lipids, association of lipids with proteins, some naturally occurring lipoproteins. The chapter on sphingolipids is by Prof. Zabin, the last two chapters by Dr. Gurd, and the rest of the book by Dr. Hanahan. Each is an acknowledged authority in his field. Discussion is limited essentially to the chemistry of the lipids present in mammals, while lipid metabolism is the subject of a companion volume.

The three authors differ greatly in style and in approach to their topics. Dr. Hanahan mentions that much of his material has been used for graduate lecture courses, and his chapters gave the reviewer an impression of expanded lecture notes with rather slipshod continuity. On the general level, it is curious to find simple glycerides discussed after the more complex phosphoglycerides, while the minor phospholipids described are all phosphoglycerides and their inclusion with these would have facilitated orderly development. On a more detailed level, there are many points at which the reader is halted by an apparently unjustified conclusion from the preceding argument, later to find the missing material. Two examples must suffice. Near the top of p. 51 "convincing evidence" of optical activity is mentioned, but the relevant data are given near the bottom of the page. About the middle of p. 188 reference is made to "the above chromotropic acid method", which leaves the reader guessing until the top of p. 189. Sometimes there is discrepancy with what has been said earlier; for example, on p. 44 it is reported that β-glycerophosphoric acid is converted quantitatively into the α-form by boiling acid, but on p. 45 we learn that an equilibrium mixture results. A section on p. 73 opens with the words "When confronted with the information cited in Chapter 1". This so-called information is simply a generalized statement repeated almost word for word on p. 73. The whole of Dr. Hanahan's material would benefit from careful editing, even to the reference points in the text for the footnotes on pp. 78, 88 and 91. It is, nevertheless, a valuable source of up-to-date information in a rapidly developing field.

The other contributions are superior in orderly approach and clarity of expression. Presumably they were specially written and not adapted from lecture material. The chapter on sphingolipids is agreeably concise. The two chapters on lipoproteins make up more than one-third of the book and are wholly admirable. Indeed, they redeem this beautifully produced book from the level of the adequate but uninspiring.

J. A. LOVERN

SCIENCE IN THE PRIMARY SCHOOL

Approaches to Science in the Primary School

Edited for the New Education Fellowship by Evelyn Lawrence, Nathan Isaacs and Wyatt Rawson. Pp. xiii+143. (London: Educational Supply Association, Ltd., 1960.) 10*s*. 6*d*. net.

WITH the recent increasing development of science in the secondary school, a growing need has been felt to prepare the way in the primary school. A committee was appointed by the New Education Fellowship to "consider the place of direct observation and experiment in the primary school".

This committee felt that the introduction of science to the curriculum could cause difficulties for the teachers, either through a feeling of inadequacy in themselves or through lack of ideas of approach, so this book was compiled for the teacher. It is not a suggested syllabus, nor is it merely a collection of articles, even though each chapter is written by a different author, including practising teachers who have contributed articles on their own approaches and experiences with different age-groups.

The book deals with the aims and the objects of starting science in the primary school. It explains that such teaching is a necessity not only for the scientifically minded but also for any child who is to grow up in this day and age.

Two chapters are entitled "Diverse Roads to Diverse Sciences": these titles speak for themselves. A further chapter deals with making the most of what one has, whether in the town or the country. Another deals entirely with teachers' needs and difficulties, and suggestions on how to overcome them. One section of a chapter offers leads for science lessons from the children's own, very commonplace, questions. There is a bibliography both for the teacher and the pupil.

The book makes interesting reading, and should act as a helpful guide to the teacher with any doubts.

B. J. G. MAITLAND

Chromatographic Reviews

Progress in Chromatography, Electrophoresis and Related Methods, Vol. 2. Edited by Michael Lederer. Pp. viii+195. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 46*s*.

APPEARING about a year after the first, this second volume contains six articles. In a preview of gas-liquid chromatography (C. J. Hardy and F. H. Pollard), principles and techniques are dealt with and there are 600 references. Despite the vast literature on the subject, the authors have succeeded remarkably well in covering the essentials so adequately. Starch-block electrophoresis (H. Bloemendahl) is a comparatively short article which provides clear practical details and reviews applications of this important method. G. Biserte and associates write about the paper chromatography of dinitrophenyl amino-acids. This is a most satisfying article, for not only does it supply full data about solvent sytems and R_F values but it also includes details of the preparation and properties of dinitrophenyl amino-acids and describes the dinitrophenylation of proteins and peptides with subsequent treatments for liberating and examining the labelled terminal amino-acids.

Flavonoid pigments are covered by J. H. Harborne, who deals clearly with the paper chromatography of the many substances belonging to this class. The article on human hæmoglobin (H. K. Prins) is a most interesting survey of electrophoretic and chromatographic techniques which have been used with such striking success in the past ten years for demonstrating and isolating a large number of hitherto unknown hæmoglobins. Finally, E. Hayek reviews a special kind of chromatography of inorganic substances based on adsorption and precipitation mechanisms. Like the first volume, all the articles are in English, and were originally published in the *Journal of Chromatography*. This second volume is full of interest, is up to date and maintains the high standards of its predecessor.

R. CONSDEN

Review of Studies in the Flow of Information among Scientists

Vol. 1: Text. Pp. v+62. Vol. 2: Tables. Pp. ii+48. (Washington, D.C.: Bureau of Applied Social Research, Columbia University, 1960.) Available from the National Science Foundation, Office of Science Information Service, Washington, 25, D.C.

THIS review, which was commissioned by the National Science Foundation, is not a review in the ordinary sense. It is rather part of the raw material for such a review. The authors have assembled the statistical material of a number of reports on the flow of information to scientists under the different questions considered. They come to no conclusions. Their purpose seems to have been to leave to others the formulating of conclusions, and only to suggest where further work might be done. The documents will be of value only as an *aide memoire* to those who are planning further surveys.

The subject of the flow of information to scientists is undoubtedly important. So far, however, the efforts to understand this process have led mainly to the collection of curious information. This has resulted in very few measures for improving the situation. The report makes no serious effort to direct attention to what might be useful and what is not. My own paper of 1948 (which is the earliest covered by the survey) is extensively quoted in the tables, although, so far as I know, it never led to any positive action except more surveys. My paper of 1958, which was a detailed survey of the use of serial literature in the Science Museum Library in 1956, is only referred to in one table. But this later paper was used to decide what literature in the Science Museum Library should be duplicated in the National Lending Library for Science and Technology.

Two important results on the flow of information to scientists which we in the Department of Scientific and Industrial Research Headquarters obtained are not mentioned at all. The first was the availability of Russian scientific literature in the United Kingdom in 1956; the second was the extent to which working scientists said they could read French, German and Russian. It matters not. The first result was mainly instrumental in starting the National Lending Library. The second assisted materially in the discussions which led to the starting of the Department of Scientific and Industrial Research's Russian translating programme. Let not the cynic say from this example that in this field "Useful results lead to action. Useless results lead to learned papers". The fundamental problem is going to become more serious as the scientific effort of the world increases.

D. J. URQUHART

Some Problems of Chemical Kinetics and Reactivity

Vol. 1. By Academician N. N. Semenov. Revised and expanded by the author. Translated by Dr. J. E. S. Bradley. Pp. x+305. (London and New York: Pergamon Press, 1958.) 50*s*. net.

PROF. SEMENOV'S pioneering book on chain reactions was published more than twenty years ago, and since then there has been intense activity in the study of chemical kinetics, of which there is now extensive literature. In the preface the author states that he does not think the main problems are completely solved. The number of papers published since 1950, quoted in the references, is very large, and the subject has been brought up to modern publications so far as possible. The Russian work is fully covered, but the literature of all countries has been included and older work of importance finds its place. The treatment is critical, and when complete evidence is lacking or findings are contradictory this is pointed out.

The first part of the book deals with radical reactions in three main branches: uniradical reaction, alternative univalent free radical reactions, and reactions of biradicals. The section on hydrocarbon oxidation reports recent work, including that of Shtern, which suggests that aldehyde formation is more important than peroxide intermediates, but it cannot be said that finality has been reached in this field.

The second and third parts deal with chain reactions. There is a critical discussion of the inhibiting effect of nitric oxide. The initiation and termination of chains on walls are considered from a new point of view, which is also involved in the discussion of heterogeneous catalysis. The appendixes deal with activation energies calculated by the London method.

The translation is very good, and the book reads as if it had been written in English originally. With the later volumes, the work is one which should be carefully studied by all physical chemists.

J. R. PARTINGTON

PORPOISES AND THE BOW-RIDING OF SHIPS UNDER WAY

By ANDREW A. FEJER
Woods Hole Oceanographic Institution and Illinois Institute of Technology, Chicago
AND
RICHARD H. BACKUS
Woods Hole Oceanographic Institution, Mass.

IN recent years there has been a controversy concerning the phenomenon of porpoises dwelling almost motionless near the bows of ships under way while obtaining propulsive power from the moving vessel. In reading the papers[1–5,7] that have been published relative to this matter it is evident that certain observational facts have been overlooked by earlier investigators. In view of this, and on the basis of some new observations made by one of us, we would like to offer an explanation of the phenomenon of bow-riding based on a model which differs from those proposed earlier.

Since Woodcock reported his observations on 'wave-riding' porpoises in *Nature* twelve years ago[1] the several papers published concerning this subject have offered explanations given in terms of the forward-sloping surface of the bow wave—hence the term 'wave-riding'. Woodcock[1] and Woodcock and McBride[2] showed that the component of the submerged weight taken along the wave slope was scarcely sufficient to balance the resistance that the animal experiences as it is moved through the water. Hayes[3] pointed out that near the wave surface the isobars are not horizontal and that the force due to the buoyancy (B) must therefore be normal to the slope of the wave (Fig. 1); consequently the hydrodynamic resistance (D) of the porpoise can be balanced by the component of the body-weight parallel to the wave surface ($W \sin \theta$). Scholander[4], on the other hand, refutes this argument on the basis of drag measurements made on small, streamlined, neutrally buoyant models that were towed in the waves of a vessel. He believes[5] that the porpoise places itself ahead of the bow wave with the tail flukes buried in the forward-facing portion of the wave (Fig. 2) in order to provide the thrust ($L_f \sin \theta$).

Observations by one of us (R.H.B.) of porpoises and their swimming and bow-riding have been distributed over the period of the past ten years, although it is only during about the last two that we have watched these charming animals with the present problem particularly in mind. These observations have been made from vessels having lengths of 100–214 ft. and speeds of 7–14 knots. None of these relatively small vessels generates conspicuous bow waves.

The animals concerned in these observations are the bottlenose porpoise, *Tursiops truncatus*; the saddleback porpoise, *Delphinus delphis*; the white-sided porpoise, *Lagenorhynchus acutus*; *Stenella styx*; and other unsuccessfully identified porpoises including several of the poorly known genus *Stenella*. The largest of these animals is *Tursiops*, which may attain a length of about 11 ft. The others are smaller, generally being, in adulthood, from six to eight feet long. These small cetaceans all belong to the sub-family Delphininae of the family Delphinidae (the 'duodecimo' whales of Herman Melville's classification). It is interesting to note that the next sort of cetacean in point of size (Melville's 'octavo' whales), the Orcinae of the family Delphinidae, most of which reach lengths of 15–20 ft. at maturity—the potheads or pilot whales, *Globicephala* spp.; the killer whale, *Orcinus*; the false killer, *Pseudorca*; *Grampus*; and the like—are not known to be bow-riders nor are cetaceans of still larger size. (The most famous of all individual bow-riders is the porpoise 'Pelorus Jack', which escorted vessels in and out of Pelorus Sound, New Zealand, during the years 1880–1912 and was granted special protection by the New Zealand Government. This animal is supposed to have been a grampus (*G. griseus*), but this is not certain. In our experience grampuses avoid close approaches to a vessel, generally not coming nearer than about 50 or 75 ft.)

Would-be bow-riders, when first observed at a distance of several hundred yards, seem to swim purposefully towards the ship for taking their stations. Sometimes porpoises thus approaching come to within 30 or 40 ft. of the ship but no nearer. On a number of occasions we have watched porpoises

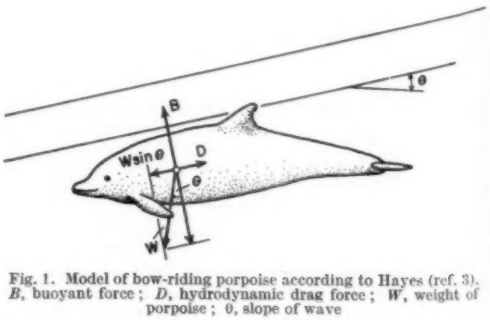

Fig. 1. Model of bow-riding porpoise according to Hayes (ref. 3). B, buoyant force; D, hydrodynamic drag force; W, weight of porpoise; θ, slope of wave

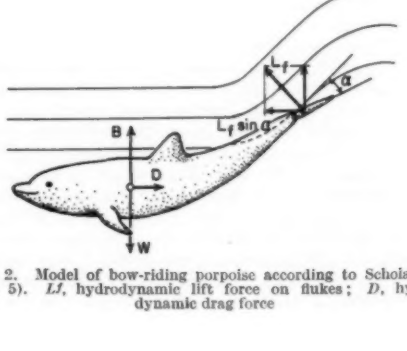

Fig. 2. Model of bow-riding porpoise according to Scholander (ref. 5). Lf, hydrodynamic lift force on flukes; D, hydrodynamic drag force

approach on a nearly reciprocal heading from that of the ship, then turn sharply on to the ship's heading when the range between ship and animal was 20 or 30 ft., and be gently overtaken by the ship as they assume a bow-riding station 1–5 ft. ahead of the ship's stem.

Once at a position at the bow the animals may remain only for seconds or for as long as an hour or more. Often a group of eight or ten porpoises may join the ship together and spend 10–15 min. riding at the bow. Then the group may diminish slowly over the period of 5 min. or so as the animals drop away by ones and twos.

The distance between the ship's stem and the porpoise's flukes is usually between less than a foot to five feet. The lateral spread of bow-riders is generally about 3 ft. to either side of the stem. Sometimes when the porpoise is riding very close to the ship and is to the side of the ship's midline the flukes may be up to a foot or 18 in. behind the ship's stem. The animals are between one or two and five or six feet below the surface and may be arranged in two or even three layers. The animals rise to the surface, of course, as it becomes necessary to breathe and often leap clear, or partly clear, of the water at these times.

While one may detect the swimming motions of porpoises at close range from the ship's side, it is often very difficult to see these motions in bow-riding animals. A novice observer often suggests that this is because of the high frequency and low amplitude of the tail beats; but it appears that it is really due to the facts that: (1) at times the porpoise is not swimming at all; (2) at times the animal is swimming easily with low-frequency and low-amplitude strokes; (3) that the common vantage point of the observer at the top of the ship's stem is a poor one for detecting the vertical swimming motions of an upright porpoise.

Often one sees individuals proceeding on a sinuous course, repeatedly swerving from right to left and back across the very bow while rolling from side to side. Bow-riders may go along on their sides or even upside down and we have seen individuals proceed while slowly revolving about their long axis. One posture assumed by the bow-rider, which we have only recently noticed, seems of particular significance. This was first observed in *Tursiops* from *Chain* on November 20 and 21, 1958, in the ocean area known as the Blake Plateau. From time to time individuals swimming on their side were seen to throw the tail into a strong downward flexure and hold it in this extreme position for two to four seconds. At these times it was hard to observe the angle of attack of the flukes but it was noticed that such porpoises were generally, if not always, so oriented that the back was towards the long axis of the vessel, that is, a porpoise on the starboard bow coasted on its left side and vice versa. It might be assumed that these animals coast in this posture while belly-down or -up; but this, of course, would be less easily seen from the top of the ship's stem. Wrinkles in the skin were noticed on several occasions when the tail was flexed downwards. These were located at the point of flexure and probably do not have a dynamic significance such as the skin folds reported for this species by Essapian[6] may have. On May 12, 1959, off Cádiz, Spain, about two dozen *Tursiops* were observed from *Chain*, and tail-down coasting the same as that described above was seen.

On May 16, 1959, just west of the Strait of Gibraltar and on May 26, 1959, in the Malta Channel this activity was observed in *Delphinus*, animals that are for the most part about 6 ft. long. The tail-down coasting posture of *Delphinus* does not appear to differ from that observed in *Tursiops*; however, it has been observed less frequently in *Delphinus* than in *Tursiops*. That is, after first noticing this bow-riding attitude in *Tursiops*, we saw it in the next two encounters with *Tursiops* but only in two of the next eight meetings with *Delphinus*. Since *Delphinus* is in general smaller and of quicker movement than *Tursiops*, the details of its motion may be more difficult to discern; on the other hand, it may assume this particular posture less frequently. Furthermore, varying states of the sea and the heading of the ship relative to the sea probably affect the behaviour of the animals. In neither species can this activity be described as continuous. Individuals can be watched for periods of many seconds riding close to the ship's bow, but assuming the tail-down attitude only at irregular intervals and then for only a few seconds at a time. On August 27, 1960, tail-down coasting was observed in an unidentified species of the genus *Stenella* from *Bear* near 38° 30′ N., 78° 00′ W. It was noted on this occasion in a porpoise riding on its side at a depth of 5 or 6 ft. in the deeper of two layers of bow-riding animals.

It is readily apparent that some of the observations reported above are not compatible with the bow-riding model proposed by Hayes[3]. The propulsive force required by the porpoise depends in that case on the magnitude of the angle θ between the streamline and the horizontal (Fig. 1). As this angle decreases exponentially with depth, the explanation offered by Hayes is clearly incomplete when the porpoise is not located in the immediate vicinity of the surface and when small and relatively slow vessels are involved that do not produce bow waves of sufficient size for wave-riding, that is, waves of slopes of 15° or more and large enough to contain the body of the porpoise. It should be pointed out, however, that hydrodynamically, Hayes's explanation, while limited in scope, appears to be correct and that the contradictory data reported by Scholander[4] seem to be the consequence of limitations pertaining to the latter's apparatus, which did not permit him to control and maintain the position and orientation of the model relative to the wave surface.

Considering next the model proposed by Scholander[5], one can determine readily that a propulsive force of sufficient magnitude can result when the animal places its tail flukes in a bow wave in the manner shown in Fig. 2. However, the objection raised by Hayes[7], that the forces and moments do not balance and that consequently the porpoise is not in equilibrium, appears to be sound. Indeed our calculations show that in Scholander's model equilibrium is not attainable: the force on the tail flukes contributes a large moment around the centre of mass and because of the shape of the porpoise and the fact that the pectoral fins are located too near the centre of mass (maximum girth is at the point of insertion of the pectoral fins) it seems impossible that the animal could generate an opposing moment of equal magnitude.

In order to arrive at a model which is consistent with our observations it is proposed to focus attention on the pressure distribution in a horizontal plane ahead of the bow. If one neglects the effect of the

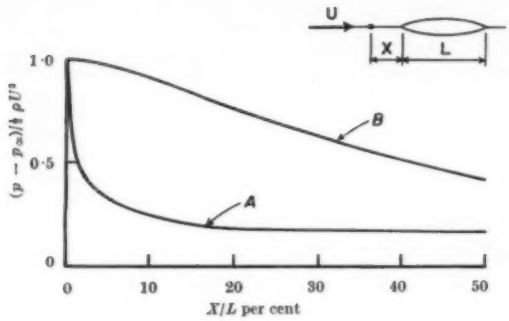

Fig. 3. Pressure distribution in a two-dimensional flow past cylindrical shapes. *A*, ship's water-line profile; *B*, circular cylinder; p, pressure at point X; p_∞, pressure at a great distance from profile; ρ, density of water; U, velocity of flow

surface and keel, that is, considers the flow to be two-dimensional, one can readily determine the pressure field ahead of the bow with the aid of potential theory. Fig. 3 shows the pressure distribution along the centre-line ahead of the bow, obtained in this manner, for a cylinder having a cross-section of the shape of an ordinary ship's water line as taken from ref. 8. For the sake of comparison the distribution for a cylinder with a circular cross-section is also shown. A streamlined body placed in this region experiences a force which is approximately equal to the negative of the average pressure gradient in the field times the volume of the body[9]. It is apparent from the pressure distribution in Fig. 3 that this force is maximum at the bow and decreases gradually as the distance between the bow and the object increases. At a location where the force due to the pressure field equals the drag experienced by the body from the flow around it, the conditions appear to be right for bow-riding. This situation was considered using the dimensions and shape of a female *Stenella styx* which was harpooned from *Bear* on July 27, 1959, at 37° 42′ N., 73° 34′ W.[10]. The resulting curves are presented in Fig. 4 showing the distance d between the bow and the tail of the porpoise as a function of the length L of the vessel for the water-line profile used in preparing Fig. 3. It should, of course, be understood that the distances shown in the

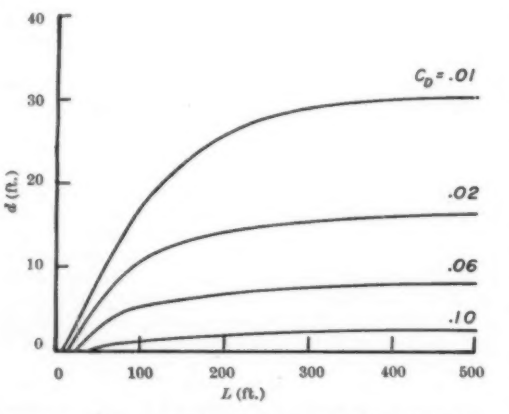

Fig. 4. Dependence of distance between tail of bow-riding porpoise and the bow on length of vessel for various values of the drag. Drag $D = 1/2\ \rho U^2 A\ C_D$; A, frontal area of porpoise; C_D, drag coefficient

figure are larger than one would expect and that this is due primarily to the assumption of two-dimensional flow. Furthermore, it should be pointed out that it is within the power of the porpoise to vary the distance by appropriate changes in posture that will influence its drag, and that the presence of vertical and lateral pressure gradients in addition to those along the centre-line will make it necessary for the porpoise to assume some of the curious positions that have been described above. These positions remind us of the attitude assumed by the sausage-like object employed by G. I. Taylor[9] to demonstrate the equilibrium position of a body in a flow field in which the streamlines are curved. In reading Dr. Taylor's paper it becomes clear that, due to the complexity of the situation, intuition will be of little help in arriving at the posture and position the porpoise must assume in order to remain motionless in the pressure field ahead of the bow.

In turning sharply on to the ship's heading for bow-riding in the manner described earlier the porpoise could, of course, be guided visually or by echo-location, but it seems most plausible that in manœuvring into bow-riding position and during the bow-riding itself, the animal is responding directly in a tactile way to the pressure field generated by the vessel.

In viewing our results it is of course necessary to realize that the situation to which our calculations pertain is a highly stylized one. In reality the porpoises are now coasting, now swimming to make adjustments in their position relative to the ship or to change their posture, now coasting again. These changes seem to be necessary to counteract fluctuations of the pressure field due to the state of the sea and the resultant motions of the ship, and to correct any mismatch between propulsive force and drag occurring, at times, as the result of a change in the animal's posture. The resulting picture is quite involved, and a more complete and accurate analysis of the bow-riding activity would require detailed information regarding the shapes assumed by the animal and the field of flow ahead of the vessel.

Recently, near 38° 30′ N., 73° 00′ W. on August 26, 1960, we observed what appeared to be the riding of wind waves five to six feet high by a porpoise of the genus *Stenella* the length of which was about 6 ft. In the best-observed instance two porpoises rode side by side in exactly the same posture for several seconds near the top of the forward slope of the wave. Little, if any, of the porpoises' bodies were exposed (possibly the dorsal fins). The heads were down and the tail portions were raised but seemingly not quite to the extent that the long axes of the bodies were parallel to the forward slope of the wave. After proceeding with the wave for several seconds the porpoises fell behind it. This observation is similar to those recently reported by Caldwell and Fields[11] of 'surf-riding' porpoises. They report that the animals rode the top front of the wave with their heads down and only their dorsal fins and a small part of their backs exposed. In these cases Hayes's model is not only appropriate but it seems to be the only feasible one. Our calculations show that even shallow wind waves having slopes of 10–18° and travelling at velocities in the vicinity of 5–6 m./sec. could be utilized for this purpose by porpoises in minimum drag posture; and based on this observation it seems not unreasonable to conclude with the speculation that in regions of the oceans where steady, strong winds are known to raise large waves that move steadily over long distances,

even large cetaceans could very well use wave-riding techniques for migratory travel.

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RADIOCÆSIUM IN HUMAN BEINGS

By Dr. J. RUNDO
Atomic Energy Research Establishment, Harwell

THE detection of the long-lived fission product, cæsium-137, in human beings in the United States in 1955 [1], was confirmed for residents of the United Kingdom in 1956 at Harwell[2] and at Leeds[3]. Subsequent measurements of body radioactivity made at the Atomic Energy Research Establishment, Harwell, have demonstrated the presence of radiocæsium in all persons, whether employed at Harwell or not. The results of measurements made in 1956 and 1957 were reported in 1958[4]; a re-examination of all the early measurements has now been made, and some conclusions about the trend of the levels to the end of June 1960 are presented here.

A description of the method of measurement has been given previously[2,4,5] and will not be repeated here, but it should be mentioned that in comparing the levels from subject to subject it is convenient to use the ratio of cæsium-137 content to potassium content. In this way the effects of variations in the sizes of the subjects and in their potassium contents are minimized, and the cæsium content is related to the mass of the muscle, its main site of deposition. In the following results the cæsium–potassium ratio is expressed in μμc. cæsium-137 per gm. potassium.

Results

(a) Subjects Resident near Harwell

The data for subjects living in Berkshire and Oxfordshire are presented in Table 1, where the mean values for six-monthly periods to June 1959, are given; since June 1959, measurements have been made in the months shown. Most of these persons are employed at the Atomic Energy Research Establishment, Harwell; none has had any occupational exposure to cæsium-137. In Fig. 1 the trends with time are shown for some individuals who have been measured more than once. In considering the significance of the difference between the mean values for any two consecutive periods, and of small (10 per cent) variations in the results for any one individual, it should be borne in mind that the standard error of counting is at least ± 4 μμc. cæsium-137 per gm. potassium, and that the groups being averaged are small. Further, up to December 1958, the groups were largely composed of different subjects, and the justification for the comparison of the mean values from those different groups is open to question. Also, the inclusion of one extreme value has a pronounced effect on the mean for the group; one subject who has only been measured regularly since July 1959 is in this category, and in Table 1 the mean values in parentheses are those including this man. Apparently, the cæsium-levels in these subjects remained substantially constant nntil about the middle of 1957, after which they increased to a maximum in July 1959. The mean value for June 1960 shows a significant fall from the December 1959–March 1960 values.

Table 1. Mean Cæsium–potassium Ratios for Subjects Resident in Berkshire and Oxfordshire

Period	No. of subjects	μμc. Cæsium-137 per gm. potassium
Feb.–June 1956	11	33
July–Dec. 1956	3	30
Jan.–June 1957	11	35
July–Dec. 1957	7 (8)	41 (46)
Jan.–June 1958	6	43
July–Dec. 1958	8 (9)	53 (56)
Jan.–June 1959	12	56
July 1959	11 (12)	63 (66)
Dec. 1959	7 (8)	56 (61)
Mar. 1960	10 (11)	58 (62)
June 1960	10 (11)	52 (55)

(b) Subjects Resident near Windscale

The accidental release of radioactivity from the No. 1 Pile at Windscale in October 1957 led to temporary increases in the radiocæsium content of milk from a wide area of England and Wales[6–9]; the biggest increases were close to Windscale[6]. Although no extensive study has been made of the levels in humans in this area, a few subjects have been measured, and the results in Table 2 illustrate the effect of the accident. The pre-accident level was 107 μμc. cæsium-137 per gm. potassium (mean for four subjects); this was 2·5–3·0 times the contem-

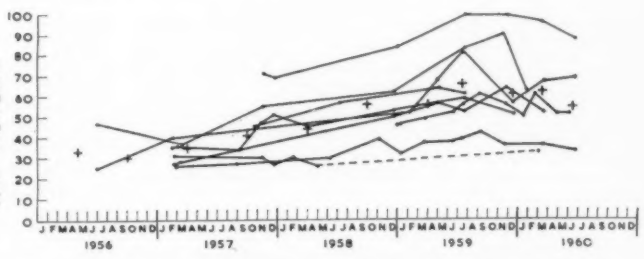
μμc. ¹³⁷Cs per gm. K
100
90
80
70
60
50
40
30
20
10
0
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1956
1957
1958
1959
1960

Fig. 1. Trends with time of the cæsium-137/potassium ratios in some individuals who have been measured more than once, compared with the average for all controls. +, Mean values for all control subjects

Table 2. Cæsium–potassium Ratios in Subjects resident near Windscale

Subject	Date measured	μμc. Cæsium-137 per gm. potassium	
		Pre-accident	Post-accident
G.	29. 3.57	138	
	26.11.58		179
C.	25. 4.57	107	
	13. 3.58		443
	21. 7.58		286
T.	27. 6.57	107	
	15. 1.58		370
	3. 7.58		227
	11. 1.60		128
Mrs. *T.*	9. 9.57	77	
*Go.**	2. 4.58		224*
*R.**	7. 5.58		183*
W.	18. 5.60		134

* These two men left Windscale on 10.1.58; their cæsium-137/potassium ratios on that date would have been somewhat higher than the values in the table.

porary average for subjects resident in Berkshire and Oxfordshire. This can be attributed in part to the higher rainfall in the area around Windscale with the resulting higher cæsium-137 levels in milk[8], and in part to slight environmental contamination from the Windscale Works[9]. For this reason the level cannot be regarded as typical for northern England. The cæsium-137/potassium ratio increased roughly four-fold to a maximum around January–March 1958 as a result of the accident, and the level fell to less than twice the pre-accident value towards the end of 1958

(*c*) *Other Subjects*

Only a few subjects resident elsewhere have been measured; the results are given in Table 3, in chronological order.

Table 3. Cæsium-potassium Ratios for Twenty Subjects with various Residences in Europe and North America

Subject	Date measured	Residence	μμc. Cæsium-137 per gm. potassium
R.	20. 4.56	California	35
H.	14. 5.56	Hampshire	33
B.	28. 9.56	Yorkshire	34
Be.	24. 6.57	London	44
L.	31. 7.57	Devonshire	79
Mrs. *L.*	2. 8.57	Devonshire	71
W.	8.11.57	Yorkshire	63
M.	8.12.58	Buckinghamshire	88
A.	8. 1.59	London	88
Mi.	22. 5.59	Illinois	75
Mrs. *Mi.*	22. 5.59	Illinois	53
Bi.	9. 7.59	Ottawa	93
Me.	17. 9.59	Berlin	73
T.	19.11.59	London	88
W.	29. 4.60	Surrey	51
Mrs. *W.*	29. 4.60	Surrey	45
An.	24. 5.60	New Mexico	53
S.	13. 6.60	Caithness	93
V.	22. 6.60	Dusseldorf	50
Mrs. *Ma.*	28. 6.60	London	56

There is a general increase in the levels during 1956–59, but the data are too sparse for any definite conclusions to be drawn or correlations to be made (for example, with levels in milk). However, it is interesting to note that, with one exception, all subjects measured in 1957, 1958 and 1959 gave values higher than the means in the corresponding periods for persons living in Berkshire and Oxfordshire.

Correlation with Milk Intake

It is known[8] that cow's milk is an important source of cæsium-137 in humans, and a correlation might be expected to exist between the cæsium–potassium ratio in humans and their daily milk consumption. The latter quantity is somewhat difficult to determine and is likely to vary from day to day. However, data on nine subjects resident near Harwell, whose milk intakes were considered to be reasonably constant and to be known, were used. It should be noted that in estimating the milk intake of the nine subjects an attempt was made to include an allowance for milk used in cooking, but not for that in a variety of manufactured foods which are of widespread distribution throughout the country. In Fig. 2 the cæsium-137/potassium ratios (measured in late 1958 or early 1959) are plotted as a function of daily milk intake; two subjects drinking 0·43 litre per day both had 46 μμc. cæsium-137 per gm. potassium. There is apparently a correlation between the cæsium-137/potassium ratio *in vivo* and the milk intake, and it suggests that at that time about 26 μμc. cæsium-137 per gm. potassium originated from sources other than milk. The mean value for all control subjects was 56 μμc. cæsium-137 per gm. potassium, so that milk was responsible for about one-half the average cæsium burden.

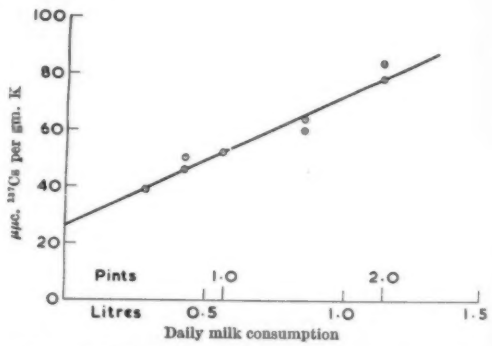

Fig. 2. Correlation of cæsium-137/potassium ratios *in vivo* with daily milk consumption

When the cæsium-137 concentration (in μμc. per kgm. body-weight) was plotted as a function of daily milk intake, a similar correlation was found but with greater scatter. Since for equal body-weights the musculature can vary considerably, and since the greater part of the body's content of potassium and cæsium is in muscle, this is not altogether surprising.

Radiation Dosage from Potassium and Cæsium-137

The radiation dosage to the gonads may be calculated if we assume that the potassium and cæsium-137 are uniformly distributed throughout the body. There is evidence that the potassium content of gonadal tissue is close to 0·20 per cent by weight[10], which is about the same as the average for the whole body (0·21 per cent by weight[4]). There are no data on the distribution of cæsium-137 in individual tissues, but there seems to be no *a priori* reason to suppose that it differs markedly from potassium, and we shall incur no great error in the assumption of uniform distribution.

Taking published values for the β-particle and γ-ray emissions of potassium[11,12], and deriving a value for the 'geometrical factor' for the γ-ray contribution from the calculations of Bush[13], with the application of the theorem of reciprocal relations[14], the dose-rates to the testes from 0·2 per cent by weight of

potassium become 17 and 2 mrads per year from β-particles and γ-rays respectively. These are in agreement with the values of 18 and 2 mrads per year calculated by Spiers[10], who used a value of 0·215 per cent for the mean potassium content of the body.

For the mean value of 63 μμc. cæsium-137 per gm. potassium (July 1959), the dose-rates from the radiocæsium are 0·53 and 0·56 mrad per year from β-particles and γ-rays respectively, it being assumed that the mean β-particle energy is 0·227 MeV.[15]. The total dose-rate to the testes is thus 1·1 mrads per year from cæsium-137 compared with 19 mrads per year from potassium, an increase of about 6 per cent. Compared with the total background radiation of approximately 100 mrads per year, the increase is about 1 per cent.

This calculation was made with the assumption that the metastable barium-137m was in equilibrium with its parent cæsium-137 in the gonadal tissue. The metabolic dissociation of barium-137m from its parent in various tissues of the rat, reported recently[16], would have only a slight effect on the calculated dose-rates. No dissociation of barium-137m was observed in the testes, but there was an excess of barium-137m in the blood. If this also applies in man, the β-particle dose-rate to the testes would be increased very slightly by the electrons from the internally converted γ-rays from the excess barium-137m in the blood present in the gonad, while the γ-ray dose-rate would not be altered significantly.

Discussion

Since the cæsium-137 content of an individual varies with the milk intake, and the concentration of radiocæsium in milk varies with geographical location[8], a comparison of the mean values *in vivo* with those obtained elsewhere is scarcely justifiable. However, it is of interest to compare the trend observed in the groups resident in Berkshire and Oxfordshire with the trends observed in the United States at the Argonne National Laboratory, Illinois, and at the Los Alamos Scientific Laboratory, New Mexico. At the former laboratory, Miller and Marinelli[17,18] have published the mean cæsium-137/potassium ratios in a group of eight to thirteen control subjects; these are set out in column two of Table 4. At Los Alamos results for control groups of 10 subjects measured in 1956, 14 subjects measured in 1957 and 37 subjects measured in 1958 have been reported[19–21] and these are included in column three of Table 4. The 1956 results are taken from Fig. 3 of ref. 19, the 1957 results are taken from Fig. 2 of ref. 20, while the 1958 results are for the 19 subjects who were measured in both the first and second halves of the year[21]. The data show that not only is the trend reported for Chicago substantially the same as that found in Britain, but the mean values are practically identical. The values reported from Los Alamos are very similar for 1956 and 1957, but are somewhat higher for 1958. However, Langham and Anderson[22] reported a mean value of 50 μμc. cæsium-137 per gm. potassium in 500 residents of New Mexico for the whole of 1958.

We may conclude that the radiocæsium found in persons employed at the Atomic Energy Research Establishment, Harwell, is a consequence of world-wide fall-out from nuclear test explosions, rather than of local contamination. Some support for this conclusion may be seen in the fact that the subject

Table 4. Mean Cæsium-137/potassium Ratios (in μμc. per gm.) in Control Groups resident in Illinois and in New Mexico (U.S.A.) and in Berkshire and Oxfordshire (U.K.), respectively

Date	Chicago, Illinois	Los Alamos, New Mexico	Berkshire and Oxfordshire
Jan.–June 1956	32	~32	33
July–Dec. 1956	—		30
Jan.–June 1957	35	~32	35
July–Dec. 1957	—	~42	41 (46)
Jan.–June 1958	46	58	43
July–Dec. 1958	51	64	53 (56)
Jan.–June 1959	61 (March)–64 (June)		56
July–Dec. 1959	57 (Sept.)–55 (Dec.)		63 (66) (July)–56 (61) (Dec.)

drinking two pints of milk daily, and with a content of 73 μμc. cæsium-137 per gm. potassium (Fig. 2) has never had any contact with radioactive material and only visited Harwell once, for the purpose of the estimation of his body content. Comparison of the mean values for all control subjects (as set out in Table 1 and plotted in Fig. 1) with either the rate of fall-out or the cumulative fall-out[23,24] at Abingdon, Berkshire, does not enable us to attribute the increase observed during 1956–59 in the levels *in vivo* to either of these parameters exclusively. However, it is clear that the decrease from the maximum level in July 1959 must be associated with the reduction in the rate of fall-out since the spring of that year[25].

With regard to the correlation between the cæsium-137/potassium ratio and the milk intake, it should be noted that there is a correlation between milk and meat consumptions which has an economic basis. Since meat contains cæsium-137, a correlation might still be found as shown in Fig. 2, even if there were no cæsium-137 in milk. For the nine subjects from whom the data in Fig. 2 were obtained there were no major differences in economic backgrounds; but the possibility should be borne in mind.

A correlation between the cæsium-137/potassium ratio *in vivo* and the daily milk consumption was also found by McNeill and Green[26] for 30 subjects in Toronto, Canada. The mean content was 51 μμc. cæsium-137 per gm. potassium in December 1958, which is about the same as found in Britain. Anderson[20] plotted the mean cæsium-137/potassium ratios *in vivo* in 1957 for different regions of the United States against the cæsium-137/potassium ratios in the milk in those regions and demonstrated a correlation; extrapolation to zero indicated that 23 μμc. cæsium-137 per gm. potassium derived from food other than milk. This is similar to the value of 26 μμc. cæsium-137 per gm. potassium deduced from Fig. 2. However, it should be noted that there are substantial differences between the average diets of residents of the United Kingdom and of the United States.

It is not clear to what extent the levels of cæsium-137 *in vivo* are controlled by the daily potassium intake, and the possible influence of naturally occurring (inactive) cæsium-133 is also unknown. That the metabolisms of cæsium and potassium are not identical is shown by the fact that the value of the ratio:

$$\frac{\mu\mu c.\ ^{137}Cs \text{ per gm. K } \textit{in vivo}}{\mu\mu c.\ ^{137}Cs \text{ per gm. K in urine}}$$

in three subjects studied by Miller and Marinelli[27] in 1956 and 1957 when the cæsium-137/potassium ratios *in vivo* were essentially constant, averaged 2·2. A value of 1·95 was found in one subject tested by

me in early 1959 when the levels *in vivo* were increasing, and a value of about 2 for the dietary discrimination factor was estimated by Langham and Anderson[22].

I am indebted to Mr. R. G. Speight for assistance in many of the measurements reported.

Note added in proof. The decrease from March 1960 of the mean cæsium-137/potassium ratio *in vivo* has been maintained. In September the value for 13 subjects resident in Berkshire and Oxfordshire was 48 μμc. cæsium-137 per gm. potassium (cf. Table 1).

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OBITUARIES

Prof. H. W. Swift

Herbert Walker Swift, emeritus professor of engineering in the University of Sheffield, died near Keswick on October 14. He was born in 1894. From elementary school he won a scholarship to Christ's Hospital and from there an open scholarship to St. John's College, Cambridge, where he read mathematics and physics. He served in the First World War, rising to the rank of captain, and was wounded and mentioned in despatches. After the War he returned to Cambridge and completed the Mechanical Science Tripos as a prizeman with first-class honours and he also won a 'half-blue' for swimming.

Swift obtained his early engineering experience with Hollins Brothers, where he became chief engineer. In 1922 he was appointed assistant lecturer in the University of Leeds. By 1926 he had published papers on several diverse topics and was awarded a D.Sc. by the University of London. For ten years he was head of the Mechanical Engineering Department at Bradford Technical College, and continued to publish in the field of power transmission and lubrication.

In 1936 Dr. Swift was appointed to the chair of engineering in the University of Sheffield, which chair then embraced the Civil, Mechanical and Electrical Departments of the University. During his period at Sheffield he was induced to take a scientific interest in the plastic forming of metal in all manner of forming processes. At a time of empiricism in this field, he made a remarkable impact in bringing logical and scientific thought to the problems involved and was the instigator of a school of plasticity which has had far-reaching subsequent influence through the work of his students.

He was responsible for the planning of both buildings and courses in the enlargement of the School of Engineering at the University of Sheffield. His ill-health brought about his premature retirement just before this task was complete.

Swift was a man of immense energy, deep insight, great enthusiasm and great kindness. He was brilliant, thorough and never mediocre. He had an unquenchable thirst for knowledge. In addition, his students will remember him as a vigorous and inspiring teacher with a readiness to appreciate their personal problems and treat them sympathetically.

J. P. Duncan

Dr. H. Zeitlinger

Heinrich Zeitlinger joined the firm of Henry Sotheran and Co. in 1894; save for a short break, he remained with it until his death on October 15, in his ninetieth year. A bookseller of distinction, he rendered notable service to science.

The volumes of Sotheran's "Bibliotheca Chemico-Mathematica" were, according to the original title-page, "compiled and annotated by H. Z[eitlinger] and H. C. S[otheran]", and on them Zeitlinger lavished the affection and erudition of the true bibliophile. Almost every famous work on the exact sciences is listed, with comments identifying and characterizing the author, indicating the content, and illuminating the treatment by apt quotation from a wide variety of authorities. An austere bibliographer might wish that these quotations had been dated, since this would have helped in tracing changes in critical estimation, but the defect is slight in such a wealth of scholarship spiced with gentle humour. Where else, for example, can one hope to learn of the only mathematical treatise written to alleviate the pangs of sciatica?

Zeitlinger's expert knowledge of Newton's work enabled him to gather together a considerable portion of Newton's library, which had been lost to sight for some two hundred years and partly dispersed. Several valuable items were recognized from annotations in Newton's handwriting, which Zeitlinger had studied with much care. It was thus possible, for

example, to identify Newton's own copy of Barrow's "Euclid", and first and second editions of the "Principia" with his manuscript revisions and additions. The collection was purchased by the Pilgrim Trust and presented to Trinity College, Cambridge, in the library of which it now rests; but for Zeitlinger's detective skill, scholarly enthusiasm and admiration for Newton, the opportunity of assembling this historic collection might have vanished for ever.

T. A. A. BROADBENT

NEWS and VIEWS

Meteorological Office: Headquarters Reorganization

THE present structure of the Meteorological Office was set up in 1957 as a result of the recommendations of the Brabazon Committee. The forthcoming move of the headquarters sections of the Office, now divided between London, Harrow and Dunstable, to Bracknell during 1961 has provided an opportunity to reconsider that organization in the light of changes which have taken place, and are likely to take place in the future, in meteorology. The division of the Office into two directorates, for services and for research, respectively, with an administrative division controlled by an assistant secretary, will remain unchanged. The balance between the research and services directorates has been improved by the creation of a new deputy director post, which has been filled by Mr. J. S. Sawyer, formerly chief forecasting research officer. He will be deputy director (dynamical research), in charge of climatological research in addition to dynamical and synoptic research. This reflects the view that forecasting work should be closely integrated with climatological investigations. The importance of the relation between the latter and long-range forecasting, in particular, is emphasized by the transfer of long-range forecasting research from the synoptic research to the climatological research branch.

Two aspects of the work of the directorate of services have increased greatly in importance since 1957. One is the rapid growth of public services, and this is catered for by the creation of an assistant director (general services) post, whose main concern will be agricultural services and the Weather Information Centres. The Climatological Services Branch will now be responsible for all climatological inquiries of a routine nature, including world climatology and marine climatology (formerly dealt with by the Climatological Research and Marine Meteorology Branches, respectively). The second development is the increasing complexity of the problems of data processing, and a new post, assistant director (support services), has been created to deal with these problems. The creation of the new branch will facilitate the development of an integrated system of data collection, processing and storage, a problem which has been the subject of much intensive work in the Office during the past few years.

Dr. J. M. Stagg, C.B., O.B.E.

ON September 7, Dr. J. M. Stagg retired from his position as director of services of the Meteorological Office. Dr. Stagg, who comes from Dalkeith, Midlothian, joined the Meteorological Office in 1926, and during 1932–33 led the highly successful British Polar Year Expedition to Fort Rae in Canada. His name soon became well known among geophysicists, and in 1939 he was appointed superintendent of Kew Observatory, where he carried out some notable work in radiation. In 1943, Dr. Stagg was made chief meteorological adviser to the Supreme Commander, Allied Forces, Europe, and it was he who briefed General Eisenhower in preparation for the *D*-day landings in Normandy in 1944. For his war-time services Dr. Stagg was made an Officer of the Legion of Merit by the President of the United States. After the War, Dr. Stagg was made principal deputy director of the Meteorological Office, and in 1957 he was appointed to the newly created post of director of services, responsible for the 'operational' side of the Office. His keen physical insight and excellent administrative qualities made him outstandingly successful in this capacity. He was created a C.B. in 1954. In the international field, Dr. Stagg has taken a keen interest in the activities of the International Union of Geodesy and Geophysics, and he was for a time general secretary of that Union. In 1955, he was awarded the Gauss-Weber Medal for his contributions to geomagnetism. It is understood that he intends to resume his work in this field during his retirement.

Dr. A. C. Best, O.B.E.

ON September 8, Dr. A. C. Best succeeded Dr. J. M. Stagg as director of services of the Meteorological Office. After graduating with first-class honours in mathematics and physics at the University College of Wales, Cardiff, Dr. Best joined the Meteorological Office in 1926 as a junior professional assistant. During his career he has had a wide experience of both the research side and the operational side of the Office, at home and abroad. His early work was in the field of atmospheric turbulence, culminating in the publication of an important Geophysical Memoir on the transfer of heat and momentum in the lowest layers of the atmosphere. His later research has been devoted mainly to cloud physics, and he has written a number of papers on the formation and size distribution of raindrops and cloud droplets. He was awarded the L. G. Groves Memorial Prize for Meteorology in 1952, and made O.B.E. in 1953. Since 1955 Dr. Best has been a deputy director on the services side of the Meteorological Office.

Zoology in the University of Delhi: Prof. B. R. Seshachar

PROF. B. R. SESHACHAR, who has been professor of zoology in the University of Mysore since 1946, has been appointed professor of zoology in the University of Delhi. After a few papers on teleosts, anura and palæontology, he worked during 1932–45 on *Gymnophiona*, describing new species and investigating their development, their germ cells and their cytology. His discovery of a homologue of Bidder's organ is perhaps not universally known. Since 1946 he has been mainly concerned with ciliate Protozoa. India can probably fill many gaps in our existing knowledge of this group. For example, Seshachar has made a

collection of Indian types of *Spirostomum* sp.; this includes different mating types which can readily be induced to conjugate, whereas this has only rarely been seen in Europe. However, his main work has been on the chemistry and physics of the macronucleus. Perhaps Seshachar's greatest service to Indian zoology was to organize a laboratory at Bangalore in which a wide variety of researches went on side by side. Apart from work related to his own, classical comparative morphology and embryology, especially of teleosts, were studied, as were the anatomy and development of *Loris tardigradus*. Endocrinological research was carried out on various Indian vertebrates, and both nuclear and extranuclear cytology were studied in a number of invertebrates. Even when the results of such work are not very novel, it is essential as a foundation for the teaching of physiological zoology in India.

Biology in the University of Malta : Dr. O. L. Thomas

Dr. Owen Lewis Thomas, who has been appointed to the chair of biology in the Royal University of Malta, was born in New Zealand and graduated in medicine at Otago University Medical School. While a lecturer in anatomy in the Department of Prof. W. E. Adams, he gained his M.D. (with distinction) in that subject and was awarded a Beit Memorial Medical Research Fellowship. In 1946, he entered Oriel College, Oxford, and studied under Dr. John R. Baker in the Linacre Department of Zoology and Comparative Anatomy, gaining his D.Phil. degree in biological sciences. In 1951, Dr. Thomas was appointed senior lecturer in physiology in St. Bartholomew's Hospital Medical School, London. His main research publications relate to the histology and cytology of the nervous system. He has recently completed his D.Sc. thesis while acting as a teacher of biology in the Napier Girls' High School.

The National Central Library

The forty-fourth annual report of the Executive Committee of the National Central Library, covering the year ended February 29, 1960, refers to disappointing progress in planning the proposed new building in Store Street (Pp. 20. London: The National Central Library, 1960). The possibility of obtaining additional storage space outside Central London is being investigated. The lending service of the Library has shown a marked tendency to expand, the number of requests for loans increasing by nearly 8 per cent, although books published since January 1, 1959, are no longer supplied to libraries borrowing through the Regional Library Bureaux. To facilitate quick communication in dealing with urgent requests, telex is being installed at the Library. The Executive has also recommended putting in hand a project involving the use of the xerographic process to deal with the arrears of material awaiting insertion in the National and Outlier Union Catalogue, in which finally the great majority of works available for loan in Britain will be recorded. The work done by the British National Book Centre on behalf of British libraries in re-allocating duplicate and other redundant books is now being extended to overseas libraries. The Executive Committee and the Trustees, after considering the Roberts Report, have reiterated to the Minister of Education their view that the inter-library lending system, with its centre at the National Central Library, should be regarded as a whole and financed half by the State and half by the participating libraries. The alterations in the Library's book selection policy are also considered (see *Nature*, **187**, 992; 1960). The percentage of applications successfully handled decreased from 76·18 per cent in 1958–59 to 72·22 per cent in 1959–60, probably in consequence of the fact that such requests increasingly relate only to books that could not be traced through the Regional Systems. Of the 76,521 separate issues from or through the Library, 12,793 were to university libraries, 8,318 to special outlier libraries, 2,299 to Government departments, 3,622 to overseas libraries, 3,299 to industrial and research organizations, 33,902 to public libraries and 10,323 to adult classes. University libraries lent 28,611 books, and 3,494 books were lent to 53 countries and 1,871 borrowed from 29 countries, the chief borrowers being Hungary (435), West Germany (352), Czechoslovakia (478) and U.S.S.R. (230), and the chief lenders the U.S.S.R. (414), France (380) and West Germany (362).

Rubber and Plastics Research Association of Great Britain

The Articles of a new combined rubber and plastics research association for the United Kingdom—to be known as the Rubber and Plastics Research Association of Great Britain—were adopted at an extraordinary general meeting of the executive committee of the Research Association of British Rubber Manufacturers in London on November 15. Seventy members of the British Plastics Federation, about a third of its membership, have already agreed to support the new Association. Speaking at the meeting, Mr. P. W. Howard, president of the Research Association of British Rubber Manufacturers, pointed out that the Research Association of British Rubber Manufacturers was engaged on work of overlapping scientific and technological interest to the rubber and plastics industries, and also on work in the thermoplastics field, much of which was of a similar nature to that envisaged by the plastics industry. The plastics manufacturers who are founder-members will be represented in the Association by a Plastics Research Committee and by representatives on the Council. They become members of the new Association as from January 1, 1961. Both industries will gain much from this joint venture. It has been announced by Mr. David Radford, chairman of the British Plastics Federation, that research will be carried out at the existing laboratories of the rubber industry, at Shawbury, near Shrewsbury, and that work has already begun on new buildings, with an area of 14,000 sq. ft., to house the additional personnel and equipment for plastics research.

Computer Centre at Woolwich Polytechnic

Sir Thomas Spencer, chairman of Standard Telephones and Cables, Ltd., and a governor of Woolwich Polytechnic, opened the Computer Service Centre at the Woolwich Polytechnic on November 22. The Centre has a *Stantec Zebra* automatic electronic digital computer, designed by Dr. W. L. van der Poel, of the Netherlands Post Office Research Laboratories, and developed and manufactured by Standard Telephones and Cables, Ltd. The machine is suitable for mathematical, scientific, engineering and statistical calculations and for commercial, financial and industrial applications. A large library of problems already programmed is available to users. The Computer Centre, which is established in the

Mathematics Department of the Polytechnic, will be used for the following purposes: (1) as a service to users in industry and commerce who are invited to discuss their problems with the computer staff and use time on the computer; a fee will be charged for this latter service; (2) for the training of students in the use of digital computers. It is intended that every student of the Polytechnic who is studying for a B.Sc. degree in mathematics, physics, chemistry, engineering or economics, and every student studying for a diploma in technology or a higher national certificate with endorsements, will have an opportunity to write programmes for and to operate the computer; (3) as an aid to research by members of the staff and postgraduate students of the Polytechnic and other educational establishments.

Liverpool Observatory and Tidal Institute

THE Liverpool Observatory and Tidal Institute, which for many years has been administered jointly by the Mersey Docks and Harbour Board and the University of Liverpool, is to become the sole responsibility of the University. The Observatory was founded in 1845 by the Liverpool Dock Trustees, and the Mersey Docks and Harbour Board, on its formation in 1858, took over the operation of the Observatory. The main reason for its establishment was the absence of knowledge at that time concerning nautical astronomy and even the most elementary details of correct determination of chronometer-rates and meteorology. The present Observatory building at Bidston was erected in 1864. The Observatory continued largely as an astronomical and meteorological institution until the death in 1928 of the then director, Mr. Plummer, an officer of the Mersey Docks and Harbour Board. For some years prior to this there had been close association with the Tidal Institute which had been founded by the University of Liverpool, largely through the influence of the late Mr. Charles Booth, a Liverpool shipowner, with whom was associated Prof. J. Proudman, and from January 1, 1929, the Board and the University agreed to combine the tidal work of the Institute and the work of the Observatory at Bidston under the name "The Liverpool Observatory and Tidal Institute", administered by a joint committee of both bodies. Since that time the joint committee has been extended to include representatives of the Admiralty and the National Institute of Oceanography. In addition to the work of tidal prediction, for which it is famous, the Institute has built up an international reputation in the scientific world and is deeply involved in the study of major problems such as the determination of mean sea-level and the investigating of storm surges as well as other matters involving geophysics and oceanography. The activities of the Institute are now far wider than was envisaged when the joint committee was set up in 1929, so the Dock Board has handed over its responsibilities to the University of Liverpool. For the time being the Observatory will be administered by the acting director, Dr. J. R. Rossiter, following the retirement of Dr. A. T. Doodson (see *Nature*, November 19, p. 625).

The Netherlands Universities Foundation for International Co-operation

THE organization known as the Netherlands Universities Foundation for International Co-operation was founded in 1952 to promote international co-operation in academic life at universities and elsewhere. It seeks to do this by functioning as a centre for consultations among institutes and persons at home and abroad; by encouraging the founding and maintaining of special forms of international education in the Netherlands; by keeping those abroad informed about university life and scientific activity in the Netherlands and vice versa; and by lending more general assistance with regard to measures and facilities on behalf of international academic and scientific contact and exchange. The Board of Governors is composed of representatives of all Dutch universities and the honorary president is H.R.H. The Prince of the Netherlands. The Foundation furthers international cultural exchanges in various ways, among others by founding institutions and courses of education on an international basis. Here it is active in a field which has been reconnoitred inadequately, and which presents many problems. It is aware of the need to subject this field of activity to further investigation. As yet, only a modest beginning has been made on this work, but individual research workers have been given commissions to form teams of experts to study comparative education; the problems of inter-cultural communications; the difficulties students from abroad encounter in adapting themselves in the Netherlands; and the evaluation of study in a foreign country. Another subject receiving close attention is European integration, the gradual achievement of which is believed to depend to a considerable extent on voluntary international co-operation within the world of higher education and research, and on the attention given to European affairs in individual institutions of higher learning. A pamphlet prepared by the Foundation describes the detailed work of the organization (Netherlands Universities Foundation for International Co-operation (NUFFIC): Aims and Activities. Pp. 16. The Hague: Netherlands Universities Foundation for International Co-operation, 1960).

The Mauritius Institute

THE annual report of the Mauritius Institute for 1958 notes the increasing demand on the library services, which include a scientific section, which has been combined with the Library of the Royal Society of Arts and Sciences to form a nucleus of works on natural history and biology (Pp. i + 27. Port Louis: Mauritius Institute, 1959. 25 cents). The report includes brief descriptions of the various sections of the Natural History Museum of Port Louis, which was reopened to the public in 1957, as well as of the Historical Museum. A function of the Mauritius Institute is to build regional collections and have them accurately identified, and the annual reports of the Institute should be particularly useful to workers on any group of the Mascarene fauna or flora. This Museum report includes some notes on research in zoology, entomology and botany, a list of publications during the year and also lists of Museum accessions, additions to the fauna of the Mascarene Islands and of library acquisitions. Similar lists are given in the annual report of the Mauritius Institute for 1959, to which are also appended a note on recent advances in the study of the fauna of the Mascarene Islands and one on the Special Collection of the Mauritius Institute Library dealing with the Mascarenes, Madagascar and the Indian Ocean (Pp. ii+28. Port Louis: Mauritius Institute, 1960. 50 cents).

Public Health and Medicine in the U.S.S.R.

The World Health Organization has issued a useful introductory guide to published material on public health and medicine in the U.S.S.R., based mainly on the holdings of the libraries of the World Health Organization and of the United Nations in Geneva (*Library News*, **13**, Supp. No. 2. Pp. 130. Geneva: World Health Organization, 1960). Works published during 1946–60 are included, but the emphasis is on those published since 1954, and on translations, surveys, etc., chiefly in English and French. The material is arranged under nine sections: guides to Soviet medical and scientific publications; periodicals (including a list of cover-to-cover translation journals); abstracting and indexing periodicals; bibliographies; directories; dictionaries and encyclopædias; administrative material; surveys; and miscellaneous material. The section dealing with administrative material is limited to publications issued in the U.S.S.R.; in the remaining sections works published in the U.S.S.R. and works published outside the U.S.S.R. are listed separately. Most of the entries are annotated and there is an alphabetical index.

U.S. Scientific Programmes in the Antarctic

The September issue of the *IGY Bulletin*, published by the National Academy of Sciences, provides a review of the status of the United States scientific programmes in the Antarctic, including many results of the International Geophysical Year and subsequent investigations, as well as descriptions of programmes in progress at the moment. The report contains contour maps showing the thickness of the Antarctic ice-cap and surface elevations; a description of the *Echo I* communications satellite, and of the long-range communications experiment for which it was designed as a 'passive' reflector of radio signals; and a summary of solar activity, geomagnetic disturbances, and decisions of the World Warning Agency during International Geophysical Co-operation 1959, just past the peak of sunspot activity reached during the International Geophysical Year; the relationship of solar activity and its associated terrestrial effects during the International Geophysical Co-operation 59 period to the 11-year-old solar-cycle is also described.

Automatic Data-processing in University Administration

Each year all the students admitted to King's College, Newcastle upon Tyne, are required to register before they start their work. The registration procedure involves the completion of a number of forms giving particulars about the student's date of birth, grant-giving body if any, place of residence, both while at home and at University, which out of a large range of courses he is intending to take, whether he is a new or returning student, an undergraduate or a graduate, and similar information to enable the University administration to have a complete record of the student, to arrange lecture room accommodation and to prepare the statistics required by various bodies. When done by hand this work occupies clerical staff for approximately one term. This year the task has been performed on the automatic digital computer in the University of Durham Computing Laboratory. The information on the forms was transferred to punched paper tape and processed on the computer. In just over two days all the lists and statistics required were produced, and the final lists distributed one week after the start of term. Further information about the tasks performed may be obtained from the Director, University Computer Laboratory, 1 Kensington Terrace, Newcastle upon Tyne, 2.

The Ecological Society of Australia

The Ecological Society of Australia was inaugurated at a meeting of plant and animal ecologists held in Canberra on August 1. The objects of the Society are similar to those of the corresponding bodies in Great Britain and the United States. They include promotion of ecological research and the publication of the results of research, facilitation of exchange of ideas among ecologists working in different fields, and encouragement of the practical application of ecological principles. Management of the affairs of the Society is vested in a Council, of which the executive officers for the current financial year are: *President*, Dr. K. H. L. Key; *Secretary*, Mr. J. H. Calaby; *Treasurer*, Mr. A. G. McArthur. The constitution provides for the annual general meeting to be held in the month of August, with a further general meeting in association with each meeting of the Australian and New Zealand Association for the Advancement of Science; additional meetings may be called at the discretion of the Council. The subscription for the current year has been set at £1. The address of the Secretary is: c/o Wildlife Section, Commonwealth Scientific and Industrial Research Organization, P.O. Box 109, Canberra.

The Institute of Physics and Physical Society: Awards

The Council of the Institute of Physics and the Physical Society has made the following awards: *Duddell Medal*, to Dr. J. B. Adams, director-general of C.E.R.N. (European Organization for Nuclear Research), for his leadership of that team; *Charles Vernon Boys Prize*, to Prof. A. W. Merrison, University of Liverpool, for his distinguished research in experimental physics; *Charles Chree Medal and Prize*, to Dr. S. E. Forbush, of the Carnegie Institution of Washington, for his distinguished work on cosmic radiation. The following appointments have also been made: *Guthrie Lecturer*, Dr. D. Shoenberg, of the Royal Society Mond Laboratory, Cambridge, to be Guthrie Lecturer for 1961; *Thomas Young Orator*, Dr. H. H. Hopkins, of the Imperial College of Science and Technology, to deliver the Thomas Young Oration in 1961.

Royal Society: Awards

Rutherford Memorial Appointment. Under the terms of the scheme to commemorate the late Lord Rutherford of Nelson the Council of the Royal Society has appointed Mr. W. J. Darcey (University of Melbourne) to be Rutherford Scholar for 1960 for three years, to carry out research in experimental nuclear physics at the Clarendon Laboratory, Oxford.

Paul Instrument Fund. Awards of grants by the Paul Instrument Fund Committee have been made as follows: £1,400 per annum for two years, in supplement of a previous grant, to Prof. G. F. J. Garlick, Department of Physics, University of Hull, for the continuation of work on solid state light image amplifiers; £2,000 in supplement of a previous grant, to Prof. D. Gabor, professor of applied electron physics at the Imperial College of Science and

Technology, and Dr. D. Jones, for additional equipment for use in connexion with the development of an electron interference microscope; £1,000 in supplement of a previous grant, to Dr. H. B. Barlow, assistant director of research, department of physiology, University of Cambridge, and Mr. P. E. K. Donaldson, technical officer, Physiological Laboratory, University of Cambridge, for further work on the development of (*a*) a device for automatically improving coding of messages, and (*b*) a diffused storage sequence engine.

The Pugwash International Conferences of Scientists

THE next two conferences in the series of Pugwash International Conferences of Scientists will be held in Moscow during November 27–December 4, and in the United States in the summer of 1961. The subject of both conferences will be "Disarmament and World Security", which will be discussed under two headings: (*a*) problems of disarmament and arms control; and (*b*) the role of scientists in the creation of a stable and peaceful world. About sixty-five scientists from a dozen countries will take part in the Moscow conference, the largest national groups being from the United States and the U.S.S.R. During their stay in Moscow, the participants will be the guests of the Soviet Academy of Sciences. The proceedings of the conference will be private, but a statement will be issued at the end. Further information can be obtained from Wayland Young, 100 Bayswater Road, London, W.2.

The Australian Mathematical Society: Summer Research Institute

THE Australian Mathematical Society has decided to hold its first Summer Research Institute at the Australian National University, Canberra, during January 3–31, 1961. Prof. T. M. Cherry (University of Melbourne) is to be the first director of the Institute, and will be assisted by Drs. H. Levey and J. Gani (University of Western Australia) as secretaries. The Australian National University has agreed to provide working accommodation for the fourteen Fellows of the Institute. Two groups, one in the mechanics of continua, and the other in probability and statistics, will be carrying out research at the Institute; four survey lectures reviewing particular branches of these fields have been arranged, but the Institute will otherwise remain fairly informal. Further information can be obtained from Dr. J. Gani, Department of Mathematics, University of Western Australia, Nedlands, Western Australia.

Symposium on National Health Institutes

A CELEBRATION will be held on December 12 to commemorate the twenty-fifth anniversary of the founding of the Istituto Superiore di Sanità of Rome. This reunion is being held concomitantly with the first international symposium on "National Health Institutes and Actual Research and Medico-sanitary Problems". The symposium is being sponsored by the World Health Organization, under the auspices of the Italian Minister of Health and the Istituto Superiore di Sanità. The topics to be discussed at this symposium will be: (1) organization of national institutes of health and their function in scientific research and in the safeguarding of public health; (2) national institutes of health and government controls; (3) active intervention of national institutes of health in contingent and specific problems. Further information can be obtained from the Istituto Superiore di Sanità, Viale Regina Elena 299, Rome.

Announcements

DR. ALBERT WETTSTEIN, head of the Biological Research of CIBA, Ltd., Basle, has been awarded the 1959 prize of the Marcel Benoist Foundation. The prize, which is awarded annually by the Foundation's administrative committee composed of federal government officials, university professors and the French Ambassador to Switzerland, is given to the Swiss scientist who has made the most useful contribution to human life during the preceding year. Dr. Wettstein received this distinction for his outstanding work in the field of steroid hormones.

DR. PAUL E. GAGNON is to succeed Dr. P. Ribeiro de Arruda (Brazil) as director of the International Atomic Energy Agency's Division of Exchange and Training of Scientists and Experts. Dr. Gagnon was governor of Laval University and also dean of the Graduate School at that University from 1940 until he joined the Agency. In 1947 Dr. Gagnon was elected president of the Chemical Institute of Canada.

PROF. M. V. LAURIE, professor of forestry in the University of Oxford, has been appointed a member of the Steering Committee of the Forest Products Research Laboratory (Department of Scientific and Industrial Research) at Princes Risborough in succession to Mr. Harry Douglass, general secretary of the Iron and Steel Confederation. The appointment is for a period of two years.

UNDER its plastics programme, Princeton University is offering a number of fellowships for engineering study and fundamental research in plastics leading to the degree of master of science in engineering. Applicants must hold a first degree in engineering or physical sciences from a recognized institution. The fellowships are valued at 1,500–2,100 dollars, plus tuition and fees. Opportunities for employment as part-time research assistants at 2,100 dollars per annum are also available to students not on fellowships. Further information can be obtained from Prof. Louis F. Rahm, Plastics Laboratory, Princeton University, Princeton, N.J.

THE University of Texas M.D. Anderson Hospital and Tumor Institute is sponsoring a symposium on "The Molecular Basis of Neoplasia" during February 23–25. Further information can be obtained from the Publications Department, University of Texas M.D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston 25, Texas.

A JOINT meeting of the Challenger Society and representatives of the Marine Laboratories (Development Commissioners' Scheme) will be held at the National Institute for Research in Dairying, Reading, during January 26–27, 1961. Further information can be obtained from Dr. L. R. Fisher, National Institute for Research in Dairying, Shinfield, Reading.

A PUBLIC conference "Education—18 Plus" is being organized by the Communist Party on December 3 at the A.C.T.T. Hall, 2 Soho Square, London, W.1. The conference will discuss the problem of university selection and expansion. Further information can be obtained from the assistant secretary, W. Alexander, Communist Party of Great Britain, 16 King Street, London, W.C.2.

PROGRESS IN AERONOMY

AERONOMY is a new word describing a much older branch of science. It was proposed as recently as 1954 by Prof. Sydney Chapman and refers to the study of the properties of the upper atmosphere and the physical processes occurring in it, particularly those due to solar electromagnetic and corpuscular radiation. That the term is already in general use indicates the rapid growth of interest in this field which has arisen out of the development of new experimental techniques (particularly rockets and satellites) and from the success of the recent International Geophysical Year.

The symposium on aeronomy at Copenhagen during July 19–22 was aimed at surveying recent advances in the subject, both experimental and theoretical, and was organized under the auspices of the International Association of Geomagnetism and Aeronomy. If a digression at this point may be permitted, it may be of interest to direct attention to the close and long-standing link between geomagnetism and aeronomy, arising through the interpretation of magnetic disturbance in terms of electric currents flowing in the outer regions of the Earth's atmosphere. As long ago as 1839, Gauss, in discussing the origin of the terrestrial magnetic field, commented as follows: "If we should look for the direct causes completely or partly in the exterior, we could . . . only think of galvanic currents. The atmospheric air is no conductor of such currents, neither is the vacuum. . . . However, the mysterious phenomena of the aurora in which, to all appearances, electricity in motion acts the main part, forbid us to deny outright the possibility of such currents only because of the uncertainty mentioned and it is, in any case, interesting to study how the magnetic effects of such currents would appear near the Earth's surface". The transition from a possibility not to be denied outright to certainty took a century. Nevertheless, as the proceedings of this symposium amply demonstrate, the mysteries of the aurora and associated phenomena in the upper atmosphere increase in fascination in step with the advances in our knowledge and understanding of them.

The reduction of orbital data of the satellites to yield atmospheric densities at heights up to 700 km. was dealt with by three contributors, D. G. King-Hele (Royal Aircraft Establishment), L. G. Jaccia and C. A. Whitney (Smithsonian Astrophysical Observatory). The method is based on the fact that the satellite loses energy, and decreases in orbital period, through collisions with air molecules; the derived densities represent a mean over about 60° of the orbit near perigee. One of the most interesting features revealed is a diurnal density variation above about 400 km., the day- to night-time ratio being of order 10 at 600 km. height. At these heights there is also a correlation between density and solar activity. S. F. Singer (University of Maryland) discussed, from a theoretical point of view, the distribution of density in the exosphere, that is, in the region where the rate of collision is so low that particles follow ballistic trajectories and some escape entirely from the Earth's gravitational influence. From an analysis of the data on satellite drag, Singer places the base of the exosphere at 530 km. for the neutral constituents. R. A. Helliwell (Stanford University) described how the properties of the outer ionosphere may be deduced from studies of 'whistlers'. These are low-frequency atmospherics which result when radiation from terrestrial lightning discharges suffers dispersion along propagation paths following the Earth's lines of force. Since the dispersion is greatest at the uppermost point on the path, electron densities out to several Earth's radii can be derived. How the composition of the atmosphere (both neutral and ionized constituents) has been studied by rocket-borne mass spectrometers was the subject of a paper by C. Y. Johnson (U.S. Naval Research Laboratory).

Solar ionizing radiations (ultra-violet and X-rays) were dealt with in papers by R. W. Kreplin (Washington), H. Friedman and R. Tousey (U.S. Naval Research Laboratory). Because of the opacity of the atmosphere in these spectral regions, rocket and satellite-borne instruments are needed to determine the incident flux of the various components. By combining the variation of observed intensity with height in a particular spectral line with laboratory determinations of ultra-violet absorption in atmospheric gases, we can deduce the variation of density with height of the constituent responsible for the absorption. Measurements of soft X-ray intensities indicate their importance as an ionizing agent in the lower levels of the ionosphere. H. Friedman pointed out that since the Lyman α-wave-length coincides with a 'window' in the O I absorption spectrum, this radiation penetrates to about 70 km. altitude and may contribute to the *D*-region ionization. Rocket measurements of *L*α made during solar flares have, however, shown no increases within a factor of two.

P. Mange (U.S. Naval Research Laboratory) and V. C. A. Ferraro (University of London) discussed the importance of diffusion in connexion with the distribution of both neutral and ionized constituents of the high atmosphere. Rocket mass-spectrometer measurements of the argon/nitrogen ratio show that these constituents reach diffusive equilibrium somewhat above 100 km., but below this level vertical mixing prevents their separation. The distribution of ionization depends on the rates of ion production and loss, and on diffusion, which is likely to be of greatest importance in the upper *F*-region. Ferraro presented some theoretical results which take account of the diffusion term and are consistent with the hypothesis that both *F*1 and *F*2 regions are produced by the same source of ionization.

A comprehensive review by A. Dalgarno (Queen's University, Belfast) of collision processes relevant to the interpretation of upper-atmospheric phenomena was followed by a number of papers on atmospheric emissions. J. W. Chamberlain (University of Chicago) has derived the total rate of emission from the different components of the airglow and from a bright aurora, using observed spectral intensities. He estimates the total input of energy contributing to a bright aurora to be in the vicinity of 400 ergs cm.$^{-2}$ sec.$^{-1}$. The altitudes of the nocturnal airglow emissions have been the subject of controversy for a number of years, and, in view of their importance in relation to the emission mechanisms, it was particularly gratifying to hear about direct rocket measurements, reported by D. M. Packer (U.S. Naval Research Laboratory). These referred to various radiations

from O_2, O, OH and Na(*D*); in all these cases the peak-emitting levels occurred between 80 and 100 km. The variations in the 5577 and 6300 Å. airglow emissions were discussed by F. E. Roach (U.S. National Bureau of Standards) and D. Barbier (Institut d'Astrophysique, Paris). According to the latter author, the 6300 Å. line from O I is the only radiation which is emitted from altitudes greatly in excess of 100 km. Of particular interest was Barbier's recent discovery of stable red arcs emitting the 6300 Å. line at the low latitude of Tamanrasset (in the Sahara). The sodium twilight-glow was the subject of a paper by T. M. Donahue (Observatoire de Meudon, France). At Tamanrasset (21° N.) there is little seasonal variation in abundance of sodium, with about $4{\cdot}5 \times 10^9$ atoms per cm.^{2} (vertical column), whereas at Haute Provence (41° N.) the abundance varies between 2×10^9 and 25×10^9 atoms per cm.^{2}.

Spectra of atmospheric emissions contain information about the temperature of the upper atmosphere, but difficulties in both measurement and interpretation are considerable. However, D. M. Hunten (University of Saskatchewan) demonstrated that a temperature profile between 70 and 250 km. can be satisfactorily constructed, using rotational temperatures from N_2^+ negative bands in auroræ and Doppler widths of forbidden lines in the night and twilight airglow.

A general scheme of solar and terrestrial disturbance was presented by T. Obayashi (Hiraiso Radio Observatory, Japan). High-energy protons (10–100 MeV.) reach the Earth several hours after solar flares associated with type-IV radio outbursts. These particles cause prolonged polar cap radio-blackouts in the region within geomagnetic latitudes 60–65°. They are followed after a day or so by the corpuscular cloud of low-energy particles travelling at a speed of the order of 1,000 km. sec.$^{-1}$. The interaction of this conducting cloud with the geomagnetic field produces the main part of geomagnetic storms. Various detailed mechanisms proposed to explain the morphology of geomagnetic disturbance and the nature of the different types of fluctuations were surveyed by E. H. Vestine (The Rand Corp., Santa Monica, California) in a paper entitled "Problems of Geomagnetism in Relation to Aeronomy". Of great interest in this connexion are the results of rocket and satellite experiments in which many of the features theoretically inferred have actually been measured; for example, the detection of the equatorial current in the *E*-region by Singer, Maple and Bowen, of the interplanetary fields by *Sputnik III* and the Soviet Moon rocket, and perhaps most exciting of all the discovery of trapped high-energy particles in the Van Allen belts. A particularly interesting finding has been the large magnetic disturbance in interplanetary space at the time of a terrestrial magnetic storm, made by the U.S. *Pioneer V*. Field values of the order of 50γ which were found may be compared with a general background field of 2γ or so.

The influx of interplanetary dust, as determined by various methods, was discussed by me. There remains a gap between the smallest particles producing observable telescopic or radio meteors, and the range of particle sizes detected by rocket and satellite investigations. Satisfactory comparison between the distribution of meteor magnitudes and the distribution in particle momentum or energy as derived from rockets and satellites requires rather more detailed knowledge of the structure of the particles, their orbital distribution, and of the nature of their interaction with the atmosphere than we possess at present.

I wish to conclude by thanking, on behalf of the participants, our Danish hosts, the chairmen of the various sessions, Prof. J. Kaplan (president of the International Association of Geomagnetism and Aeronomy) and Prof. M. Nicolet (chairman of the Association's Committee on the High Atmosphere) for arranging and conducting such a stimulating and rewarding meeting. The papers contributed will shortly appear in the *Annales de Géophysique* and afterwards as a bound volume which, it is hoped, will be available at a price to suit the purse of individual research workers in this rapidly expanding field.

T. R. KAISER

LATTICE DEFECTS AND THE MECHANICAL PROPERTIES OF SOLIDS

THE second symposium of the Fifth Congress of the International Union of Crystallography was held in the Guildhall at Cambridge. It opened with an introductory lecture on August 20 by Prof. N. F. Mott, who surveyed the development of our knowledge about lattice defects and their relation to mechanical properties, and outlined outstanding problems in the field. The symposium, organized by Dr. P. B. Hirsch, occupied August 22–24, and about one hundred papers were presented by contributors from eleven countries. On two days the sessions continued (with a substantial audience) until 10 p.m.; the supposedly free afternoons were, of course, usually fully occupied by private discussions. One may criticize meetings of this kind for the mental indigestion and exhaustion they produce, and argue that fuller discussion on a more restricted subject is to be preferred. Nevertheless, these larger gatherings do have a value of their own in presenting a broad picture of current progress in many associated fields, and in providing an opportunity for the assembled specialists to assess the impact of related developments on their own particular interests.

The papers of the symposium were grouped under twelve main headings, and it is clearly not possible in the present short review to refer to the significant content of all contributions. Summaries of the papers are printed in the Congress Abstracts of Communications, and the papers will appear in full in the usual scientific journals.

Four sessions were devoted to "Yield Strength" and "Work-hardening", which are perhaps the central problems of the field. Ideas on work-hardening are still in a state of flux, and discussions took place on a number of mechanisms which may be important in determining the flow stress: the stresses due to dislocation groups (Seeger); the debris left behind by moving dislocations (Gilman, who discussed the

velocity of dislocations as a function of stress); also, in a new theory, the associated accumulation of defects by dislocations as they move (Hirsch, Warrington and Hirsch); and the interactions arising from the intersections of dislocations with one another (Baird and Gale, Saada, Hale and McLean). The last authors, and also Howie, and Bailey and Hirsch, reported detailed correlations between observations on thin films and macroscopic treatments and measurements in various metals; and flow-stress measurements were discussed by Christian, Masters, Mitchell and Pargeter, and by Basinski. Other topics treated were the theoretical strength of metals (Richards) and dislocation reactions in body-centred iron (Crussard), while considerable attention was given to the mechanical behaviour of ionic crystals (Newey and Pratt; Pratt; Silverstone and Pratt; Westwood) and of semi-conductors (Bell and Bonfield; Suzuki, Kayano and Watanabe; Bernard, Berth, Burgeat, Devaux, Mme. Soula and Tsoucaris). In the sessions on "Stacking Faults" and "Twinning", two papers were concerned with the direct measurement of stacking fault energies (Howie and Swann) and associated calculations (Bilby, Grinberg and Swinden), and a number of others described the behaviour of faults and twins as observed in thin films and under stress in the electron microscope (Phillips; Price; Fourie, Weinberg and Boswell; Catlin, Walker and Lawless; Pashley and Presland). The last authors also directed attention to fine defects ('black death') produced, probably indirectly, by the electron beam. Two papers discussed the theory of twinning (Christian; Venables) and others treated kinking in cadmium (Blasdale, King and Puttick) and in alkali halide crystals (Mme. Klassen-Nekludova), and the interplay of twinning and slip in iron (Sleeswyk) and silicon iron (Hull).

In contrast to the situation in work-hardening, the survey of probable mechanisms of "Fracture" could be given (Cottrell) without provoking instant controversy. The influence of minute surface imperfections was emphasized in papers on the Joffe effect (Stokes, Johnston and Li) and on the fracture of glass (Murrell). The former authors also illustrated in another paper the interrelation of fracture and deformation in magnesium oxide. Other papers treated the fracture of zinc (Barnby and Pratt) and silicon iron (Cuff), the fracture of thin metal films (Fujiwara and Yoshida, Fisher and Nutting), and the propagation of cracks (Mme. Shaskolskaya) and the influence of inhomogeneities on this process (Bethge and Schmidt) in alkali halides. The session on "Fatigue" was opened by Thompson, and included reports on thin-film studies of the defects produced (Partridge and Segall), the role of cross-slip (Broom) and a theory of the dependence on temperature (Hirsch and Segall). Grosskreutz described experiments on crack propagation in single crystals.

There were two sessions on "Dislocations and Point Defects"—one on "Quench Hardening" and one on "Climb and Creep". The effects of irradiation and/or cold work and the course of subsequent low-temperature recovery (usually assessed by resistivity measurements) were reported for several materials: in copper and its alloys (Pritchard and Pratt, Sosin), in molybdenum and niobium (Johnson, Peacock and Wronski) and in titanium and zirconium (Smith and Stagg). Effects of straining and ageing on the mechanical and electrical properties and the etching behaviour of alkali halides were also discussed (Slifkin, Kabler and Layer; Curien and Mihailovic; Bethge and Fröhlich; Davidge, Whitworth and Pratt), and an extension of the general theory of strain-ageing reported (Bullough and Newman). Changes in impurity concentration around precipitates at dislocations and other sites in doped silicon were revealed directly by the formation of annular p–n junctions made visible by etching (Newman, Wakefield, Willis and Bullough). Internal friction studies on cold-worked gold and copper (Hasiguti and Okuda) and on quenched aluminium (Gelli and Federighi) were reported, as well as the influence of pressure and plastic deformation on point defects (Lazarus) and their contribution to stored energy and volume changes (Åström). Quench hardening was introduced by Maddin, and a thin-film study of the vacancy clusters produced in aluminium reported by Westmacott, Barnes, Smallman and Hull. The role of solute atoms and vacancies in the ageing of aluminium alloys was studied in thin films (Nicholson), by calorimetric and resistivity measurements (Borelius and Larsson), and by small-angle X-ray scattering (Gerold and Schweizer). An introductory paper on climb and creep (Friedel) was followed by a direct thin-film investigation of the shrinkage-rates of dislocation loops in zinc by climb (Price). An example of the direct and subtle experiments now possible was afforded in a paper by Dash, who was able to argue that climb observed in silicon due to the introduction of gold was due to vacancy deficiency, since left-hand screw dislocations formed right-hand helices. In similar vein, Barnes and Mazey showed that certain dislocation loops in copper foils induced by α-particles were formed by condensed interstitials, since in a foil where the particles come to rest the loops grow during the precipitation of helium bubbles, which is a process requiring vacancies. Other papers in the two sessions on "Radiation Damage" included work on copper and other face-centred cubic metals (Blewitt, Coltman, Jamison and Redman; Silcox; Makin, Whapham and Minter; Seeger, Mader and Essmann), on graphite (Williamson and Baker; Fujita and Izui) and on fission in uranium steel (Baker and Nutting). A general discussion of dislocation-point defect interaction was given by Sosin.

The session on "Recrystallization" and related phenomena included thin-film investigations on silver (Bailey) and work with other techniques on a titanium molybdenum alloy (Schofield and Bacon) and on aluminium (Weissmann, Imura and Nakayama; Fujiwara and Takano). Grain-boundary motion in sintering was discussed by Horustra and van Bueren. In the session on "Alloy Hardening", A. Kelly reviewed the yield strength of alloys, and thin-film observations on the dislocations in ordered alloys were discussed in two papers (Pashley and Presland; Marcinkowsky, Brown and Fisher). Work was also reported on the order and precipitation hardening in a nickel (24 per cent), chromium (15 per cent), aluminium (3·5 per cent) austenitic steel (Blackburn and Nutting), and of dislocations and precipitates in an austenitic steel containing niobium (Arrowsmith and Nutting).

The final session, on "Martensitic Transformations", was introduced by Read, who also discussed the plasticity of metals undergoing polymorphic transformation. Again, important work using the thin-film technique is revealing here the fine details of the transformation process. P. M. Kelly reported thin-film work on high- and low-carbon steels and on

iron–nickel alloys, and suggested correlations between the twinning and different morphologies observed and the martensite hardness. Venables has worked on thin films of stainless steel transformed by cold work, where stacking faults, regions of hexagonal ε-phase and regions of body-centred cubic α′-phase can be seen. Other papers in this session described extensions to the crystallographic theory (Wechsler and Otte) and etch-pit investigations of dislocations in the austenite (Reed and Greeson).

Thus, the subject of defects in crystals continues to expand at an ever-increasing rate, particularly under the stimulus of the development of experimental techniques permitting direct observation of dislocation lines, of which the dominant one is now the use of the electron microscope to examine thin metal films by transmission. With these high-resolution studies, our knowledge of the detailed behaviour of individual dislocations and other lattice defects is increasing rapidly, and the next ten years will see a great extension in work of this kind. While possible differences in the behaviour of defects in thin films and bulk specimens must always be borne in mind, it is clear that much valuable information about the basic mechanisms controlling bulk mechanical properties will be obtained in this way. Such information must indeed play an essential part in the solution of the more difficult problems concerned with the development of theories about the bulk mechanical properties of practical interest. B. A. Bilby

CO-ORDINATION CHEMISTRY

A SYMPOSIUM on Co-ordination Chemistry with an international attendance was held in Prague during September 5–8. It was organized by the Czechoslovak Chemical Society and by the Faculties of Inorganic Chemistry of the Charles University and the Technical University and sponsored by the Czechoslovak Academy of Sciences.

The introductory paper by Prof. A. A. Grinberg (Leningrad) consisted of a survey of the development of co-ordination chemistry in the past decade and a general review of the present position of the subject. This was followed by a general discussion of the applications of polarography by Dr. A. A. Vlček, a member of the Polarographic Institute at Prague. Dr. Vlček emphasized, in particular, the importance of searching for new complexing agents which would permit the electro-deposition of a wider range of metals than can at present be utilized in this way.

The other scientific meetings were grouped into three sections, concerned with modern preparative methods, with the physico-chemical properties and structure of complexes, and with direct methods of structure determination, respectively. Prof. S. Herzog opened the first main session with a paper on the properties of low-valency co-ordination compounds of metals with dipyridyl and phenanthroline. He described some remarkable substances, for example, zero-valent *tris*-dipyridyl derivatives of titanium, scandium and even niobium. The existence of such compounds and of magnesium and lithium dipyridyl derivatives was shown to raise a number of important problems in valency theory which were discussed in some detail. Whether these compounds are to be regarded as true low-valency compounds of the metals, or as complexes of the dipyridyl anion radical, was the interesting question which emerged in discussion.

Among the contributed papers one can mention only a few. Prof. E. O. Fischer (Munich) described the preparation of a variety of mixed diene-carbonyl complexes of zero-valent metals and discussed some very remarkable rearrangements which they undergo. Prof. I. I. Chernaev (Moscow) described his extensive work on the nitro complexes of tetravalent platinum. Dr. K. Isslieb (Jena) described a large number of novel complexes of transition-metal ions with primary and secondary phosphines. Complexes of tri- and tetra-dentate arsenic ligands were discussed by a group from Oxford.

The section on physico-chemical methods was opened by Dr. L. E. Orgel (Cambridge), who gave an elementary account of the principles of ligand-field theory and of modern methods of determining the spatial distribution of unpaired *d* electrons in transition-metal complexes. This was followed by an entertaining paper by Dr. L. G. Sillen (Stockholm), who summarized the extensive research of his school on the varied hydrolysis reactions of metal ions in aqueous solution.

Madame B. Jezowska-Trzebiatowska (Warsaw) described the use of optical and magnetic measurements in discussing the electronic structure of a variety of co-ordination complexes. The *trans* effect in a variety of complexes of divalent platinum, trivalent cobalt and divalent copper was treated in a series of papers.

A further group of papers dealt with applications of polarographic methods. Prof. K. B. Yatsimirskii (Kiev) described ingenious methods of using the catalytic polarographic currents to study complexes in solution; Miss D. Wagnerova (Prague) discussed the polarographic reduction of ruthenium nitrosyls, and Dr. E. Fischerova described similar work on trivalent chromium complexes.

The final section was opened by Prof. Porai Koshitz (Moscow), who gave a concise summary of the present state of our knowledge of the environments of metal ions in co-ordination compounds as determined by direct X-ray studies. There followed a series of papers describing structural studies on individual compounds. One may mention in particular papers on the periodate of trivalent copper, on the dipyridine adduct of the dimer of copper acetate, and on $(NH_2.CH_2.CH_2.NH_2)\,CuBr_2$. Another paper in this series by Czech crystallographers discussed the mechanism of the substitution reactions of cupric compounds.

The meeting as a whole provided an excellent opportunity for the exchange of ideas and information between scientists of all countries. For those from the Western countries it was a particular pleasure to meet and talk with their opposite numbers from Eastern Europe. Apart from the main scientific business of the meeting, foreign delegates were most hospitably entertained—a tour of the medieval city of Prague, an excursion to neighbouring points of interest and the various social activities in the evenings were greatly enjoyed by those present. L. E. Orgel

ADMISSIONS TO BRITISH UNIVERSITIES

THE second report of an *ad hoc* committee of the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom on Procedure for Admission of Students (pp. vi+31. London: Association of Universities of the British Commonwealth, 1960. 2*s*.) deals first with the action taken by the universities on some procedural recommendations of an earlier report published in May 1958 by an *ad hoc* committee. Although the new system has been in operation for only one full year, it is already clear that a marked improvement has occurred. Indeed, 86 per cent of heads of schools answering the independent inquiry of the Joint Committee of the Four Secondary Associations about delays by universities were satisfied that there was no unreasonable delay, and 66 per cent were satisfied with the decisions themselves. Throughout the universities, all those concerned are showing the utmost willingness to co-operate in making the selection and admission of students as simple, fair and rapid a process as possible. As all those concerned, in universities and schools, become more accustomed to the new procedures, the improvement should continue, and to this end the Committee recommends that the normal date for the receipt of applications should continue to be January 31 of the year in which entry is desired (the preceding November 30 for medicine, etc.); constant care should be exercised to preserve standards of precision in letters making offers of places, and the great importance of making as many early decisions as possible is specially emphasized. The Committee also reviews some continuing problems such as multiple interviews and clearing houses, and recommends that experiments should be tried, worked by volunteer professors in limited fields such as chemistry, physics, biology, medicine, dentistry, veterinary science, agriculture and law. Finally, it recommends that the admission of students generally be kept under review. The Committee of Vice-Chancellors and Principals has already invited this *ad hoc* committee to continue its consideration of future problems.

Figures for applications and admissions for 1959 show that applications to university institutions in England, Wales and Scotland were 117,300, 7,600 and 9,100, respectively, and the corresponding admissions to first-degree courses were 14,960, 1,650 and 3,720, respectively. As between the main categories, applications were distributed as follows: arts, 50,900; pre-science, 40,700; technology, 21,700; medicine, 13,100; dentistry, 4,500. The ratio of applications to the number of places available varies widely between individual universities; for example, from 6·0 to 11·4 in England, 3·8 to 6·2 in Wales, and 1·9 to 3·0 in Scotland, but no clear pattern, according to age or type of institution, emerged from more detailed examination of the figures.

Appended to the report is a brief guide for the sixth-former entitled "Getting into a University", containing the minimum information which any intending candidate needs before discussing matters with those who will give him advice and before completing his application.

INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES

R. LEVEQUE was appointed by Unesco to undertake a new mission to the Galapagos Islands. The purpose was to consolidate the basis for the establishment of a research laboratory, the primary objective of the Charles Darwin Foundation. He has now presented a report which contains a wealth of information about the destruction being wrought on the exceptional fauna of the archipelago*.

In his inventory of the present state of the animal populations, Levêque recalls that the sea-lion has succeeded in holding its own in face of the depredations of fishermen, but the fur-bearing seal (*Arctocephalus australis*) is prey to exploitation which will soon lead to extinction if drastic measures are not taken. Biological study of the species must be a priority in the programme of research.

Concerning the bird populations, the maintenance of which is bound up with the survival of the shrub cover of the islands, Levêque is less pessimistic. The larger species call for attention: albatross, flightless cormorant, penguins and especially the flamingo, which is in keen demand for its feathers, and consequently vulnerable.

The author has nothing essential to add to the excellent material on reptiles previously compiled by Mr. Eibl-Eibesfeldt. Wild pig destroy the eggs of the sea iguana, but apart from this the latter species is practically without enemies; the land iguana is holding its own.

By far the most threatened species is the giant tortoise; the local position is desperate. The massacres continue, with the destruction of eggs by feral pigs, by trading shells, and extraction of oil. It appears necessary to destroy the feral pig if the tortoise is to be saved. Levêque calls for the establishment of a large park to be fenced off with barbed wire, and gives suggestion for its boundaries.

Crayfish are being captured on a reckless scale; a single boat recently brought in three tons of crayfish tails in a few days; a thorough study of the biology of the species is required. Likewise, overfishing of the bacaleo will soon result in a serious depletion of this species.

The author lays strong emphasis on the disastrous consequences resulting from the introduction of exotic species to the Islands; dog, donkey, but especially pigs, "the worst enemy of the indigenous

* *Bulletin of the International Union for Conservation of Nature and Natural Resources*, **9**, Nos. 1–2 (1960).

agriculture, flora and fauna", and feral goats, which are on the increase and destroy numbers of plants. It would seem advisable to encourage unrestricted hunting of these animals; the fate of the Galapagos Islands, otherwise, will be similar to that of the Mexican island of Guadeloupe. Levêque emphasizes the mistake of prohibiting hunting of feral animals introduced to the Islands. The result is contrary to the objective pursued; the defenceless native fauna, the destruction of which does not require the use of fire-arms, pays the consequences and serves as food for the settlers.

A number of sound recommendations conclude the survey: engagement of an experienced botanist to undertake methodical inventory of the plant species represented; careful study of the biology of the land tortoise, and, later, of both iguanas, *Amblyrhynchus* and *Conolophus*; efforts in the fields of oceanography and meteorology resulting in a marine research scheme and the establishment of climatological stations.

INDUSTRIAL HEALTH

THE annual report of the Chief Inspector of Factories on Industrial Health in 1959 follows the pattern of the two previous separate reports, and this year includes a special chapter on "Dusts and Their Effects on the Lungs"*. The establishment at headquarters of the new pathological laboratory permitted a substantial increase in the number and variety of tests, and the combined chemical and pathological work has made possible the examination of an increasing number of workers. The Chief Inspector again emphasizes his anxiety to foster the liaison between the medical branch of the Inspectorate and members of the medical profession generally, which he regards as of great importance if due weight is to be given to significant occupational factors in the health of work people. The 532 cases of industrial poisoning or diseases, notified under the Factories Act, 1937, and the Lead Paint Act, included 64 non-fatal cases of lead poisoning, 6 cases (1 fatal) of anthrax and 25 non-fatal cases of aniline poisoning; 226 notified cases of epitheliomatous ulceration included 9 fatalities, and there were 192 cases of chrome ulceration, while 14 of the 206 gassing accidents were fatal.

The First Aid in Factories Order, 1938, was superseded by the First Aid Boxes in Factories Order, 1959, on January 1, 1960, which brings the contents more into line with modern practice than under the 1938 Order. Interdepartmental discussions on the general principles of first-aid treatment were attended by independent medical experts nominated by the Medical Research Council and members of the Advisory Panel in Ophthalmology were consulted on the first-aid treatment of eye injuries, while at the two meetings during the year of the Industrial Health Advisory Committee the possibility of developing and extending medical supervision in factories was the main subject discussed.

Publication of a new booklet, "Toxic Substances in Factory Atmospheres", was approved. The special chapter on dusts and their effects on the lungs points out that it is now becoming increasingly doubtful whether it is safe to assume that any finely divided dust is harmless if inhaled in sufficient quantity over a sufficient period. Certain dusts, too, may produce an acute inflammatory response in the lungs when they are breathed, and others, usually after many years, may bring about a cancerous development in some part of the respiratory tract. The chapter outlines some general principles on measures required to protect workers against inhaling injurious dusts as well as briefly summarizing existing knowledge about the action of particular classes of dusts.

* Ministry of Labour. Annual Report of the Chief Inspector of Factories on Industrial Health, 1959. Pp. iii+60. (Cmnd. 1137.) (London: H.M. Stationery Office.) 3s. 6d. net.

X-RAY DIFFRACTION STUDIES OF THERMALLY DECOMPOSED PERMANGANATES

By P. J. HERLEY and Dr. E. G. PROUT

Chemistry Department, Rhodes University, Grahamstown, South Africa

THE thermal decomposition of crystals of silver[1], potassium[2], rubidium[3], cæsium[4] and barium[5] permanganates in high vacuum yield pressure-time plots which are sigmoid in form. The plots for the decomposition of crystals of potassium permanganate (13·6 mgm.) and silver permanganate (20·6 mgm.) at 217° C. and 110° C., respectively, are shown in Figs. 1 and 2. The acceleratory and decay portions of the curves for the above permanganates, except silver permanganate, are described by the Prout–Tompkins equation[2]:

$$\log [p/(p_f - p)] = kt + c$$

where p_f is the final pressure. The plots for the decomposition of silver permanganate obey a modified form of the equation[1], namely:

$$\log [p/(p_f - p)] = k \log t + c$$

These equations follow if it is assumed that at the commencement of the acceleratory period strain exists in the crystal at the interface between the product formed prior to acceleration and the undecomposed material. This strain produces micro cracks in the reactant surface, and the reaction progresses inwards by a mechanism of branching planes of reaction. Mechanical disruption of the crystal, as in the case of potassium permanganate, is a consequence, not the cause, of branching. Silver permanganate, for example, does not disintegrate during reaction. It is of interest, therefore, to examine the Laue photographs of potassium permanganate and silver permanganate at different stages during decomposition when lattice distortion would be expected for both substances. Such distortion will be shown by asterism of the diffraction spots. Fig. 3, *A*, *B*, *C*, *D*, *E*, *F*, *G* were taken at 40 kV. and 20 m.amp. using

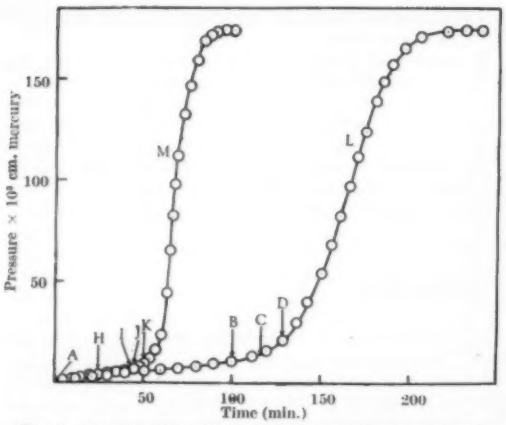

Fig. 1. Pressure-time plots for decomposition of potassium permanganate at 217° C. Curve *L*, unirradiated crystals; curve *M*, γ-ray irradiated crystals. Laue photographs taken at times of interruption indicated by arrows. Fig. 3, *A*, *B*, *C*, *D* and Fig. 4, *H*, *I*, *J*, *K* correspondingly lettered

copper radiation filtered through iron. The silver permanganate crystal was mounted about the needle axis. The crystal of potassium permanganate was similarly mounted before disintegration, but after this there was no known orientation. The corresponding times of interruption of the reactions are indicated in Figs. 1 and 2.

Asterism is not exceptionally pronounced immediately prior to acceleration, but with both silver and potassium permanganates there is a marked increase in the lattice distortion immediately after this.

Fig. 4, *H*, *I*, *J*, *K* are Laue photographs of pre-irradiated potassium permanganate (γ-ray dose in spent fuel facility at Harwell, 10 Mrads; $p - t$ plot in Fig. 1) under the same conditions as for Fig. 3. The Laue photograph of undecomposed irradiated material does not differ in any marked respect from that for undecomposed unirradiated potassium permanganate (Fig. 3*A*). It is seen that the amount of strain is extremely high at the end of the induction period. This is in accord with the suggested mechanism of decomposition of pre-irradiated potassium permanganate[6], which presumes that at the end of the induction period there are centres of decomposed material throughout the crystal and that these produce a high degree of strain in the solid.

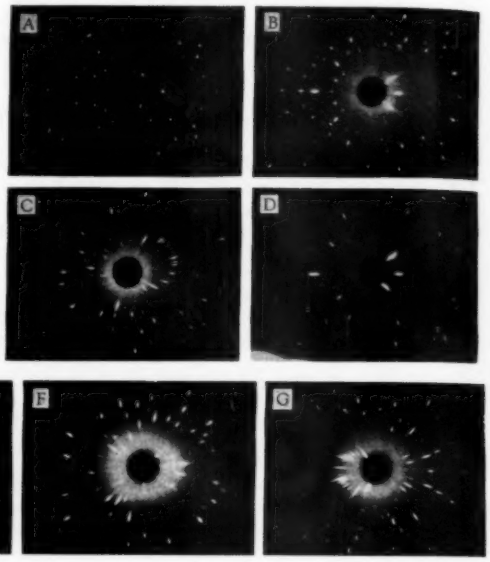

Fig. 3. Laue photographs of unirradiated potassium permanganate and silver permanganate. See legends for Figs. 1 and 2

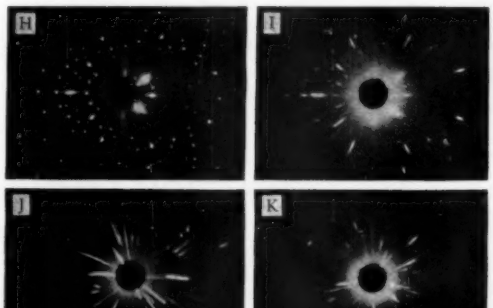

Fig. 4. Laue photographs of pre-irradiated potassium permanganate. See legend for Fig. 1

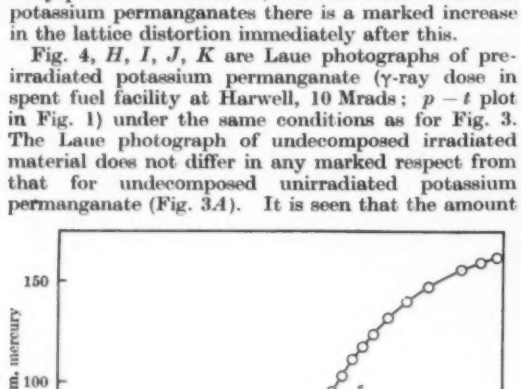

Fig. 2. Pressure-time plot for decomposition of silver permanganate at 110° C. Laue photographs of Fig. 3, *E*, *F*, *G* taken at corresponding times of interruption shown by arrows

More detailed X-ray studies of unirradiated and irradiated permanganates are in progress, and the results will be published elsewhere in due course. We wish to acknowledge financial assistance from the Council for Scientific and Industrial Research (South Africa) and for a scholarship held by one of us (P. J. H.).

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A CONNEXION BETWEEN P_c AND THE F REGION

By Dr. H. J. DUFFUS

Canadian Services College, Royal Roads, Victoria, British Columbia

THE two most promising theories of the origin of geomagnetic micropulsations of the P_c type are: (1) they originate as standing hydromagnetic waves within the ionosphere[1], or (2) they originate as standing hydromagnetic waves in the outer atmosphere[2].

Each of these theories has at least one major difficulty to overcome. The first must explain how phase stability can be produced over distances large compared with the thickness of the ionosphere; and why the modulation envelope of P_c wave trains should often be so similar at places as widely separated as College, Alaska, and the Borrego Desert in California. The second must explain how the electromagnetic field appears to be propagated through the lower ionosphere without serious attenuation; indeed, how there can be a maximum of activity on the sunlit side of the Earth where the shielding has been thought to be greatest. This paradox is the main subject of this communication.

White[3] has published a curve showing the high harmonic content of the diurnal variation of the ionospheric electron density near the equator. Afanasieva[4] has reported a similarity between P_c and f_0F_2 (although measured at different places), and a high correlation (0·75–0·97) between P_t amplitude and f_0F_2. On the other hand, Benioff[5] reports that his type A oscillations (0·3–2·5 sec. period, and thus not P_c) occur only when the electron density in the F region is unusually low.

These conflicting reports suggested that, averaged over a year, there might be a world-wide relationship between P_c and ionospheric electron density $N(h)$ which is obscured by local effects. A preliminary study of this relationship is now possible since the publication of Miss Hutton's data on the diurnal variation of P_c on the equator[6], and those of Jacobs and Sinno for the International Geophysical Year[7]; together with the most recent results of $N(h)$ during the International Geophysical Year by workers at the Cavendish Laboratory (Croom, S., Robbins, A., and Thomas, J. O., private communication) and by Schmerling[8].

As yet there are only enough data to suggest that the diurnal variation of P_c is inversely related to the diurnal variation of the true electron density at 280 km.

Figs. 1*a* and *b* respectively show the ratios of the 12-hr. and 8-hr. harmonic components to the 24-hr. component of the diurnal variations of P_c occurrence, and of $N(h)$ at 280 km. over stations covering a large range of geographical latitude. The symbols for the stations are shown along the top of each diagram.

It will be noted that the 8-hr. and 12-hr. ratios are comparable for both P_c and $N(h)$ at all latitudes, although there is considerable dependence on latitude. The harmonic vector lies in the quadrant indicated below each point relative to the 24-hr. vector.

Three different zones are suggested: (1) an equatorial zone, where the corresponding P_c and $N(h)$ vectors are in opposing quadrants; (2) a middle zone, where the effect of the ionosphere is not clearly displayed by this treatment; (3) an auroral or polar zone, where the 8-hr. vectors are in phase, although the 12-hr. vectors oppose one another.

Unfortunately, only the behaviour at the equator is reliably indicated by the present data. The harmonic content in the middle latitudes is so small that slight errors in the data would cause large phase changes, and the high-latitude data are only available from stations in opposite hemispheres.

The simplest interpretation of the equatorial behaviour is that the source of P_c oscillations lies above the ionosphere. At the surface of the Earth the amplitude is reduced by conventional ionospheric

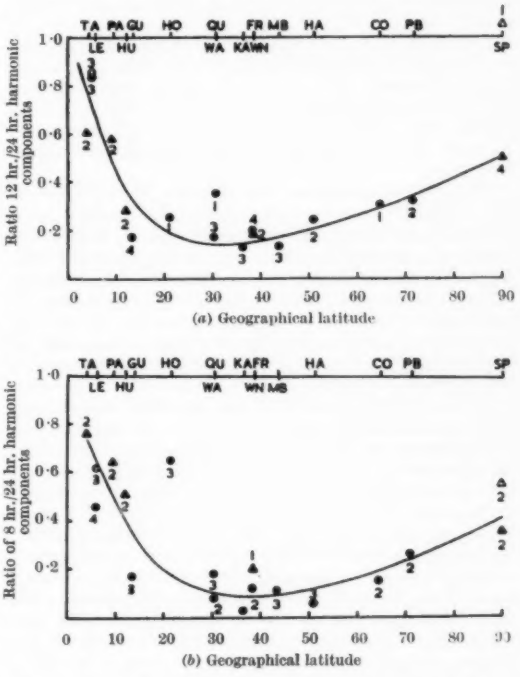
TA PA GU HO QU FR MB HA CO PB
LE HU WA KAMN SP
Ratio 12 hr./24 hr. harmonic components
1·0
0·8
0·6
0·4
0·2
0 10 20 30 40 50 60 70 80 90
(a) Geographical latitude
TA PA GU HO QU KAFR HA CO PB SP
LE HU WA WN MB
Ratio of 8 hr./24 hr. harmonic components
1·0
0·8
0·6
0·4
0·2
0 10 20 30 40 50 60 70 80 90
(b) Geographical latitude

Fig. 1. *a*, The ratios of the 12-hr. to the 24-hr. harmonic components of the diurnal variation of the occurrence of P_c (dots) and of $N(h)$ at 280 km. (triangles), averaged over the International Geophysical Year, for stations the symbols of which are found at the top of the figure and geographical latitudes at the bottom. The hollow circle is Miss Hutton's (ref. 6) May–July 1957 data. The hollow triangle is Knecht's (ref. 10) f_0F_2 data for the South Pole. *b*, The ratios of the 8-hr. to the 24-hr. harmonic components of the diurnal variation of the occurrence of P_c and of $N(h)$ at 280 km. at a number of stations. The symbols are the same as in Fig. 1*a*

shielding as discussed by Ashour and Pryce[9]. The rather complicated diurnal and latitudinal variations of $N(h)$, together with the too simple assumption of a uniform external disturbance field, have hitherto obscured the ability of their theory to explain the role of the ionosphere in the diurnal variation of P_c.

At high latitudes the 12-hr. phases of both $N(h)$ and P_c are opposed, as they are at the equator. However the 8-hr. components are in phase. Possibly this is due to occasional incursions of solar corpuscles, themselves the source of the hydromagnetic disturbance of the outer atmosphere, which are confined to the auroral zones. It must be noted, however, that not only are the polar data from different hemispheres but also that Knecht[10] observed 3-hr. phase differences between the 12-hr. and 24-hr. components of the F_1 layer and their counterparts in the F_2 layer. Thus considerably more data are required to clarify polar behaviour. Despite the occasions mentioned by Afanasieva[4] and Campbell[11] when the ionization increases during P_c events, a demonstrable effect of the polar ionosphere appears to be that of shielding.

On this basis one may venture to predict that a daily minimum in P_c occurrence will be found corresponding to the daytime maximum in ionization reported by Croom, Robins and Thomas[12], centred on 70° N. during December 1957, and in the southern hemisphere in July. The remarkable absence of P_c during the polar night noted in the International Geophysical Year literature[4,13] may be associated with this anomaly the origin of which is not understood.

The fact that P_c are found at night at both the equator and at the poles, and can have coherent phases over a thousand kilometres[14], suggests that they can extend simultaneously over the whole surface of the Earth, but with varying amplitude. If this is so, why are they so seldom seen at night in the middle latitudes ?

The simplest explanation appears to be that as the average summer month electron density is a maximum at about 20° latitude[15], the P_c field penetrates the local $N(h)$ minimum at the equator. The $N(h)$ diurnal variation is very large at the equator, and it controls the P_c-level. It does not appear to be necessary to postulate a P_c source which is highly dependent on latitude, although Kato and Watanabe's[2] expression for the outer atmospheric oscillation can have this property.

It is necessary, however, to postulate a source of P_c in the outer atmosphere which is stronger on the sunlit side of the Earth in order to explain the generally low level of night-time P_c. Scholte's study[16] of the propagation of hydromagnetic waves associated with giant pulsations concludes that energy from a rotational transverse disturbance is propagated along the magnetic field without attenuation, and hence is confined to the originating field lines. However, a part of the irrotational disturbance can act to build up a resonance along the magnetic field, and this part falls off inversely as the distance from the source of the disturbance. Thus energy from a localized source travelling with the Sun would be propagated weakly to the dark hemisphere. The amplitude of this 24-hr. component is much greater than the amplitude of the 8-hr. and 12-hr. 'shielding' components in middle latitudes. However, Figs. 1*a* and *b* show that they become comparable where the electron density exhibits particularly large diurnal variations, that is, at the equator and at the poles.

The present results do not exclude the possibility that localized hydromagnetic resonance in the upper F region is the source of some P_c disturbances, although the outer atmospheric source seems necessary to explain their large geographical extent. Local oscillations are necessary to explain the phase-changes and differences in wave-form sometimes observed over distances of a few kilometres[5,13,17]. One would expect them to be excited according to Lehnert's[1] theory.

The equivalent current system for a number of P_c calculated by Jacobs and Sinno[7] show the disturbance to be concentrated in the auroral zone. Such a picture would be consistent with P_c originating as a hydromagnetic resonance in the auroral F region, and being propagated through the F region to lower latitudes as an increasingly attenuated Alfvén wave. The occurrence of P_c at night on the equator rules out this type of propagation as attenuation would have occurred in the middle-latitude part of the path. Again, we are drawn to the outer atmospheric oscillation as the mechanism producing P_c.

Matsuchita[18] has shown that in general the electron density decreases during the main phase of a magnetic storm, although what happens to the total ionization is not yet clear. A decrease in ionospheric shielding would explain why P_c are generally of larger amplitude during such times, without postulating a change in their source energy. As Matsuchita's effect is dependent on latitude, further study of present data should clarify this point.

If the data of Figs. 1*a* and *b* are re-plotted on geomagnetic co-ordinates the similarity between P_c and $N(h)$ disappears. This suggests that the principal attenuation occurs in the lower ionosphere, where there is greater symmetry about the geographical equator. The damping of hydromagnetic waves in the ionosphere depends on the density of the neutral particles as well as on the charged particles. Thus the lower part of the ionosphere should be more effective as a shield than is suggested by its electron density alone. However, the diurnal variation of $N(h)$ is greater in the upper ionosphere. Further study of $N(h)$ data at different heights is required to clarify this very important question of symmetry.

If P_c were produced at the same heights as the S_q currents, one would expect them both to be governed by the ionospheric conductivity, and hence by the symmetry of the geomagnetic field. Thus, on the basis of the present data, I am led to discard the S_q current theory of Terada[19] and to accept a higher source for P_c.

The above arguments show that further documentation of the relationship between the occurrence of P_c and $N(h)$ (better, perhaps, the integrated density over the whole ionospheric column) may rule out one or more of the postulated sources of P_c. In particular, the study of the variations of the upper F region will help decide the question of how local oscillations occur.

The data on which Figs. 1*a* and *b* are based are rather scanty. The magnetic data are averaged from October 1957 to September 1958. The $N(h)$ data, although covering the same period, are derived only from ten International Quiet Days in each month, together with Regular World Days of the International Geophysical Year. Furthermore, the $N(h)$ curves pertain only to the 280-km. level because the ionosonde records are most complete for this height. Even so, a number of extrapolated values have been

used, especially in the late evening hours. Only four $N(h)$ stations are complete. The data from the South Pole are only for the month of September 1958[8]. Knecht's[10] values for f_0F_2 are also shown for comparison. They are averaged for the whole year.

There are large seasonal variations in both $N(h)$ and P_c occurrence. Miss Hutton's[6] P_c data from Legon show that in the summer the 12-hr. component is smaller than the 8-hr. component, although it is larger averaged over the whole year. Similarly the 1939 and 1944 $N(h)$ records from Huancayo show seasonal reversals. The ratios are shown in Table 1. There appear to be even larger changes from year to year, and for this reason care has been taken in this communication to compare data only from the same year.

Table 1. RATIOS OF 12-HR. TO 8-HR. COMPONENTS

$N(h)$	1939	June	1·9	March	0·71
	1944	June	1·6	March	0·59
P_c	1957	May–July	0·72		
	1957	October to			
	1958	December			1·8

I have not distinguished between P_c occurrence and P_c amplitude variation in this discussion, nor considered the effect of the U.T. component of their daily variation. Furthermore, the P_c data include a range of periods which several authors[4,5,13] would now treat as containing different classes of P_c.

Nevertheless the present data have indicated that P_c are less frequently observed when the electron density at 280 km. is high, providing a P_c source with 24-hr. periodicity is postulated.

This work was done at the Cavendish Laboratory, and the help of J. A. Ratcliffe, Dr. R. Knecht, Dr. J. O. Thomas, Miss A. Robbins and Mrs. S. Croom is gratefully acknowledged. It was supported by a grant from the Defence Research Board of Canada.

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REGENERATION OF INSULIN ACTIVITY FROM THE SEPARATED AND INACTIVE *A* AND *B* CHAINS

By DR. G. H. DIXON* and DR. A. C. WARDLAW
Connaught Medical Research Laboratories, University of Toronto, Toronto, 4

IT is generally accepted that when insulin is split into its *A* and *B* chains by breaking the disulphide bonds, hormone activity is abolished[1]. Recent reports[2–4] have indicated, however, that preparations of the isolated chains may possess low levels of activity, but the possibility has not been excluded that traces of intact insulin might be responsible for the effects observed. A variable factor in this work has been in the mode of cleavage of the disulphide bonds. Nicol[2] used the performic acid method of Sanger (which converts the cystine sulphur into the stable sulphonic acid, cysteic acid) and found low activity in both chains. In contrast, Langdon[4] employed reductive cleavage followed by conversion of the resulting sulphydryl groups to phenylmercuric mercaptides. He found that the substituted chains were inactive, but the preparation of *B* chain developed hormonal activity on removal of the mercury compound with thiol. This suggested that the free sulphydryl form of the *B* chain carried the hormonal activity, and it was postulated that insulin might be converted to this form at its site of action.

The present work was started with the object of cleaving the disulphide bonds of insulin by a method which would give the two chains as stable derivatives, readily separable from each other and from unmodified insulin. These requirements were met by the sulphite method of Swan[5], which yields the *A* and *B* chains as S-sulphonates ($R.SSO_3^-$). These derivatives can be separated readily by ion-exchange chromatography and purified by paper ionophoresis. It was of interest, in the light of Langdon's report[4], to convert the S-sulpho chains into their free sulphydryl forms by treatment with excess thiol and to examine the products for insulin activity. Experiments have shown that the chains as the S-sulphonates are inactive, either separately or mixed, which rules out the possibility of the preparations being contaminated with intact insulin. When the S-sulphonates were separately incubated with excess thiol, cysteine or mercaptoethanol, to convert them to the sulphydryl forms, again no insulin activity was detected. However, incubation of a mixture of the two chains with excess thiol gave rise to definite insulin activity. Since this activity had an ionophoretic mobility similar to that of intact insulin and could be neutralized with a specific anti-insulin serum, it appears that a resynthesis of insulin from the separated chains had taken place.

Preparation of the A and B chains. Ox insulin (1 gm.) was dissolved in 20 ml. of a reagent containing 0·2 *M* sodium sulphite, 0·08 *M* cupric sulphate and 8·0 *M* urea, which had been adjusted to *p*H 10·2 with ammonia. As judged by ionophoresis, these conditions gave complete splitting within a few

* Present address: Department of Biochemistry, University of Toronto.

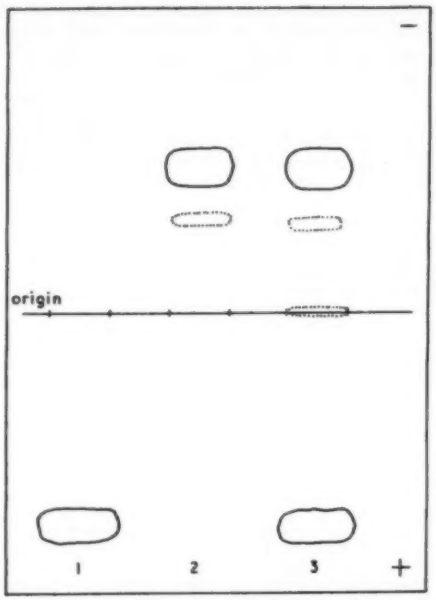

Fig. 1. Tracing of an ionophoretogram of S-sulpho *A* and *B* chains of insulin. The paper (Whatman No. 3) was wetted with a buffer consisting of 1 ml. of pyridine, 10 ml. acetic acid, 48 gm. urea and water to 100 ml. Ionophoresis was for 60 min. at 75 V./cm. in an apparatus cooled with 'Varsol *DX*3641' (Imperial Oil, Canada). After drying the paper, the urea was washed out with 95 per cent ethanol. The components were stained by spraying with 1 per cent diazotized sulphanilic acid in 5 per cent sodium carbonate, when the *A* and *B* chains appeared as bright orange bands. 1, S-sulpho *A* chain; 2, S-sulpho *B* chain purified as described in the text; 3, impure S-sulpho *B* chain prepared by unmodified procedure of Mycek *et al.* (ref. 6). The minor component in 2 has been shown to be derived from S-sulpho *B* chain during elution with 1 *M* ammonia and is likely to be a desamido derivative produced by the alkaline *p*H

minutes. The solution was left at room temperature for 2½ hr., then acidified with formic acid to *p*H 3·2 and the mixture of chains precipitated by adding 10 volumes of 95 per cent ethanol. The chains were fractionated initially by the procedure of Mycek, Clark, Neidle and Waelsch[6]. However, when the preparations were examined by high-voltage ionophoresis on paper, it was found that although the *A* chain appeared pure, the *B* chain contained appreciable amounts of *A* chain (Fig. 1). The contamination of the *B* chain with *A* chain was thought to be due to adsorption of *A* chain on to the *B* chain which was insoluble in the formic acid solution used by Mycek *et al.* When the procedure was modified to include 8 *M* urea in the buffer[7], the *B* chain stayed in solution. Paper ionophoresis of *B* chain prepared in this way showed that it was free of *A* chain (Fig. 1). The modified procedure is as follows: 100 mgm. of the chain mixture was dissolved in 2·0 ml. of buffer solution containing 2·6 *M* formic acid and 8 *M* urea and applied to a 2·2 × 9 cm. column of 'Dowex-50*X*2' (200–400 mesh) in the H^+ form which had been equilibrated in the same buffer. The *A* chain, which was not retained by the column, was eluted and then precipitated, together with urea, by adding excess acetone. The urea was redissolved by adding a few drops of ethanol. The *A* chain flocculated on standing overnight. The *B* chain was eluted from the resin with 1 *M* ammonia in 8 *M* urea and appeared at the alkaline front, leaving the copper behind as a blue band at the top of the column. Urea was removed from the *B* chain solution by passage through a 'Sephadex *G*-50' column equilibrated with 1 *M* ammonia and the *B* chain was precipitated by acidification with formic acid.

Treatment of S-sulpho chains with thiols. *A* and *B* chain preparations were treated with thiol to remove the sulphonate and regenerate the free sulphydryl group as shown:

$$\text{Chain—S—SO}_3^- + R.S^- \rightleftharpoons \text{Chain—S—S—}R + SO_3^{=} \quad (1)$$

$$\text{Chain—S—S—}R + R.S^- \rightleftharpoons \text{Chain—S}^- + R\text{—S—S—}R \quad (2)$$

The reaction was carried out by making a 50 mgm./ml. solution of the chain preparation in a 0·02 *M* formate buffer, *p*H 3·5, containing 8 *M* urea, and transferring 0·02 ml. (1 mgm.) into a reducing system made up in freshly boiled water. The total volume was 1·0 ml., and the final concentrations were: chain preparation, *c.* 0·00033 *M*, *tris*, 0·025 *M* and an amount of thiol (0·0062 *M*) which was a 20-fold excess with regard to the molarity of the chain; the final *p*H was 8·5–9·0. After standing for 30 min. at room temperature the mixture was diluted into a balanced salts solution, 'medium *A*'[8], for biological testing. In biological tests under anaerobic conditions (see below) the medium *A* was gassed with nitrogen before the reduced chains were added to it.

Biological tests. The preparations were examined for insulin activity by the mouse convulsion test, using the rotating cylinder apparatus of Young and Lewis[9], and also by a sensitive *in vitro* procedure using mouse hemidiaphragms. The latter test, which is described fully elsewhere[8], is based on the ability of insulin to stimulate the synthesis of glycogen by muscle tissue incubated in a modified Gey and Gey medium. To test the sulphydryl forms of the chains it was desirable that anaerobic conditions be used, and advantage was therefore taken of the observation that pre-exposure of hemidiaphragms to insulin, at 0° under nitrogen, sensitized the tissues so that they exhibited an insulin effect when afterwards incubated under the usual aerobic conditions of the test. The pre-exposure was done by adding three hemidiaphragms to each reaction tube, which contained 5 ml. of test solution under nitrogen. After 15 min. the tissues were transferred to vessels containing 3 ml. of modified Gey and Gey medium which was gassed with 95 per cent oxygen + 5 per cent carbon dioxide. The tubes were incubated for 90 min. at 37°, when the tissues were taken out and each hemidiaphragm analysed for glycogen by an anthrone method[8]. With each batch of hemidiaphragms, usually 30 per batch, control tests were made to determine the basal level of glycogen synthesis during incubation and also the responsiveness of the tissues to different levels of insulin.

Activities of A and B chain preparations. In early experiments with chain preparations obtained by the unmodified procedure of Mycek *et al.*, it was shown that neither the S-sulpho *A* nor S-sulpho *B* chain possessed insulin activity. However, when the S-sulpho *B* chain was treated with a thiol, cysteine or mercaptoethanol, insulin activity appeared (Table 1). This seemed at first to confirm Langdon's report[4] that *B* chain in the sulphydryl form has insulin activity. But ionophoresis of the S-sulpho *B* chain preparation revealed that it contained appreciable quantities of S-sulpho *A* chain (Fig. 1), and when this

Table 1. Activity of the *A* and *B* Chains of Insulin compared with Intact Insulin in a Mouse Hemidiaphragm Test

Preparation	Concentration of chain (μgm./ml.)	Insulin effect* ± *S.E.*	No. of hemidiaphragms
S-sulpho *A*	40	11 ± 5	6
S-sulpho *B* (crude†)	40	1 ± 4	12
S-sulpho *A* + thiol	40	10 ± 5	12
S-sulpho *B* (crude†) + thiol	40	61 ± 7	18
S-sulpho *B* (crude†) + thiol	10	21 ± 3	12
S-sulpho *B* (purified‡) + thiol	40	6 ± 5	12
S-sulpho *A* + *B* + thiol	40 + 40	82 ± 6	18
" " "	13 + 13	75 ± 10	6
" " "	4 + 4	46 ± 6	6
Insulin	0·4	74 ± 6	6
"	0·2	49 ± 5	18
"	0·04	23 ± 4	15
"	0·008	6 ± 4	12
Thiol control	—	4 ± 4	15

*Percentage increase in the amount of glycogen synthesized by the experimental tissues over that synthesized by the control tissues.
† Prepared by unmodified procedure of Mycek *et al.* (ref. 6).
‡ Prepared by modified procedure described in the text.

was removed by the method described, the S-sulpho *B* chain no longer showed activity in the presence of thiol (Table 1). Next, the significant observation was made that when S-sulpho *A* chain was added back to the purified S-sulpho *B* chain and the mixture treated with thiol, there was a reappearance of insulin activity in a yield of 1–2 per cent. These experiments were all made by the pre-exposure technique using anaerobic conditions although it was found later that the activity was still present when tested under aerobic conditions. These aerobic tests were made by diluting the chain mixture plus thiol into 'medium *A*' (1·0 ml.), oxygenating and adding 1 ml. 'medium *B*' [8] to complete the incubation mixture. Mouse hemidiaphragms were added, and, after gassing with 95 per cent oxygen + 5 per cent carbon dioxide, were incubated for 90 min. at 37° prior to glycogen analysis. In this test, as in the pre-incubation test, the yield of insulin activity from the chains was in the region of 1–2 per cent.

The *A* and *B* chain preparations were also tested by the mouse convulsion method and showed the same pattern of results, that is, no activity of the separated chains in either the S-sulpho or sulphydryl forms, but reappearance of activity (1–2 per cent yield) in a mixture of the S-sulpho chains plus thiol (Table 2).

Table 2. Activity of the *A* and *B* Chains of Insulin compared with Intact Insulin in a Mouse Convulsion Test

Preparation	Dose of chain per mouse (μgm.)	No. of mice convulsing / No. of mice injected
S-sulpho *A* + *B*	50 + 50	1/36
S-sulpho *A* + thiol	50	0/36
S-sulpho *B* + thiol	50	2/36
S-sulpho *A* + *B* + thiol	50 + 50	55/72
Insulin	1·6	56/72

Nature of the regenerated insulin activity. It seems probable that the regenerated insulin activity is due to the resynthesis of insulin itself from the separated chains. It was thought possible at one stage that the regenerated activity, observed after thiol treatment, might have come not from separated chains but from insulin molecules in which only one of the inter-chain bridges had been broken by the original sulphite treatment. However, this seems to be excluded by the finding that ionophoretically purified preparations of *A* and *B* chain remain inactive when they are treated with thiol separately, but develop activity when they are treated with thiol in a mixture. This finding would also appear to exclude activity being due to complexes such as *A*-*A*, *B*-*B*, *A*-mercaptoethanol or *B*-mercaptoethanol, although it is possible that each of these species is formed during the thiol treatment.

Evidence of a more direct nature that the regenerated activity in the reduction mixtures was due to insulin itself was obtained by ionophoretic and immunological tests. The ionophoresis was done in starch gels prepared with a buffer containing 8 *M* urea, 0·05 *M* formic acid and 0·01 *M* sodium hydroxide at *p*H 3·7 which was known to give good resolution of insulin (Smithies, O., personal communication). When a portion of the gel corresponding with a marker of bovine insulin was dialysed to remove urea and extracted with acid-alcohol, insulin activity was detected. Adjacent portions of the gel tested in this way were inactive.

The immunological tests were made with an anti-insulin serum prepared by immunizing guinea pigs with ox insulin in Freund's adjuvant, as described by Moloney and Coval[10]. The serum neutralized the hormone activity of ox and other mammalian insulins but not that of the codfish. The potency of the serum in a mouse hemidiaphragm test[8] was such that 1·0 ml. neutralized 600 milliunits (24 μgm.) of insulin. It will be seen from Fig. 2 that 0·06 ml. serum was required to neutralize the insulin activity present in a mixture of 40 μgm. each of S-sulpho

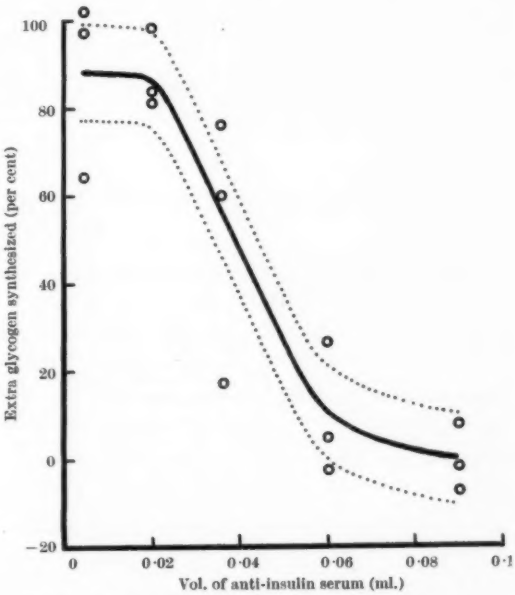

Fig. 2. Neutralization of reformed insulin activity by specific anti-insulin. The figure shows the relative amounts of glycogen, above the mean basal level, synthesized by individual hemidiaphragms incubated (in groups of three) in mixtures of increasing volumes of anti-insulin with constant aliquots of a solution containing 40 μgm. S-sulpho *A*, 40 μgm. S-sulpho *B* and mercaptoethanol. The dotted lines are at ± 1 *S.E.*

$A + B$ chains, treated with thiol and oxidized with air. This indicates that from the 80 μgm. of chain mixture 1·44 μgm. insulin was produced, which corresponds to a yield of 1·8 per cent. The fact that there is such close correspondence in the estimates of yield by hemidiaphragm tests with and without antiserum indicates that the resynthesized material has an immunological specificity indistinguishable from that of ox insulin. Had the immunological specificity of the molecule been changed, then larger quantities of antiserum would be required for neutralization (as is the case with codfish insulin[8]) and the estimates of yield given by tests with and without antiserum would differ. It is likely, therefore, that the resynthesized activity is due to a small amount of material, identical in its folded structure with insulin, rather than to a large amount of material of altered structure and lower activity.

Since resynthesis of insulin appears to occur in mixtures of separated chains, it is essential that before isolated chain preparations, particularly in the sulphydryl form, are tested for insulin activity, they be free from cross-contamination with the other chain. Preparations which met this criterion appeared to be without significant activity (<0·1 per cent of the same weight of insulin) at the levels tested (Table 1).

Mechanism of resynthesis. It was shown by starch-gel electrophoresis as described above that the isolated S-sulpho chains, on treatment with excess thiol, lost their negatively charged sulphonate groups and were converted quantitatively into more positively charged species which are interpreted as the sulphydryl forms (equations 1 and 2). One mechanism of disulphide bond formation would therefore be the recombination of the reduced chains in the presence of oxygen. An alternative mechanism of resynthesis could be the result of a displacement of a sulphonate group on one chain by the sulphydryl group on another:

$$\text{Chain—S}^- + {}^-\text{O}_3\text{S—S—Chain} \rightleftharpoons \text{Chain—S—S—Chain} + \text{SO}_3^{=} \qquad (3)$$

No final decision can be reached as to which mechanism of resynthesis is operating here, but in experiments carried out with anaerobic solutions, to minimize oxidation, the yield of insulin activity was the same as when the solutions were deliberately oxidized before testing. It must be realized, however, that even when the test solutions are anaerobic, the freshly dissected diaphragm possesses an undetermined content of oxygen. However, there is no evidence that the diaphragm exerts any specific catalytic influence on the resynthesis.

The yields of insulin obtained are not considered to be unduly low in view of the large number of possible molecular species which could be produced by random reformation of disulphide bonds in a system as complex as a mixture of A and B chains with thiol. Experiments are in progress to determine whether the disulphide bridges tend to reform in certain preferred orientations, as with ribonuclease[11], and whether recombination can be influenced so as to increase the yield of insulin. However, even with the present yield, it can be said that if chemically synthesized A and B chains were available in mgm. amounts it should be possible to obtain insulin by the above method and thereby provide the terminal step in the total synthesis of a protein with biological activity.

We wish to thank the Insulin Testing Department for the mouse convulsion tests.

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SEMICONDUCTIVITY IN PROTEINS AND HÆMOGLOBIN

Enhanced Semiconductivity in Protein Complexes

DRY proteins show a very high resistivity and energy-gap, typical values being $10^{16}\,\Omega$ cm. (20° C.) and 2·8 eV.[1,2]. Decreases in this resistivity value by a factor 10 have been achieved by H atom bombardment[3], following a suggestion to convert the protein into a free radical by chemical means[4]. We have now found that a very large effect may be achieved, following an analogy with the effect of chloranil in forming highly conducting complexes with donors such as dimethyl aniline[5,6] and phthalocyanine[7]. An aqueous solution of bovine plasma albumin was shaken with *p*-chloranil, the excess *p*-chloranil filtered off, and the solution evaporated to form a bovine plasma albumin–chloranil complex film. Such a film contained a very small amount of chloranil, approximately 0·5 per cent, corresponding to 3 chloranil molecules to 1 molecule of bovine plasma albumin. This plasma–chloranil film in the dry state had a room temperature resistivity of $3 \times 10^{12}\,\Omega$ cm. and energy gap of 1·06 eV., compared with values for a control dry film of bovine plasma albumin of $8 \times 10^{17}\,\Omega$ cm. (extrapolated) and 2·80 eV. Thus the chloranil has raised the specific conductivity of the protein by a factor 3×10^5. The bovine plasma albumin–chloranil complex showed a weak but definite electron resonance signal, with a *g*-value of 1·998 and a line-width of 6 gauss. There was no optical absorption or photoconductivity at a wave-length corresponding to the energy-gap of 1·06 eV. Bromanil and iodanil complexes gave very similar results. Admission of water vapour at a vapour pressure chosen to give approximately a Brunauer–Emmett–Teller monolayer over the (relatively small) available surface of the bovine plasma albumin–chloranil complex gave a twelve-fold increase in resistivity. This is in marked contrast to the effect of water vapour in decreasing the resistivity of pure dry proteins, including bovine plasma albumin.

These results may be explained on lines well known for inorganic semiconductors. The chloranil molecules are taken to accept electrons from the protein, creating positive holes in its conductivity band[8]. On the other hand, water is an electron donor, and by neutralizing positive holes lowers the conductivity of the bovine plasma albumin–chloranil complex. Ammonia gas shows a similar electron-donor behaviour to water, as would be expected. The twelvefold increase in resistivity is consistent with a filling up of the positive holes (10^6 increase) followed by a donating of electrons into the conductivity band (10^5 decrease). It seems probable, therefore, that in the living cell proteins may show *N* (negative) or *P* (positive) behaviour, depending on their environment, whether aqueous or lipoidal, presence of electron acceptors, free radical sources, etc. It is possible that further work will show that such changes in conductivity may result in steady-state values sufficiently high to be significant for models of electron-transport[1], carcinogenesis[9] and other phenomena in the living cell.

K. M. C. Davis
D. D. Eley
R. S. Snart

Semiconductivity in Hydrated Hæmoglobin

Hæmoglobin is regarded as a model for a large class of oxidation enzymes, where electron mobility may be of biological significance[10]. In the dry state its very low specific conductivity, about 10^{-18} Ω^{-1} cm.$^{-1}$ at 30° C., is attributed to electron and hole mobility[1] in the CO . . . . HN bridge system[8]. Water is adsorbed on natural, or methanol-denatured, hæmoglobin, corresponding to a Brunauer–Emmett–Teller '*BET* monolayer volume' V_m of 319 moles/10^5 gm. protein, whereas a close-packed monolayer of water molecules over all the molecular surfaces throughout the crystal would involve 1,160 moles/10^5 gm. protein[11]. The molecular dimensions for the latter calculation are taken from X-ray data[12] which also show that water molecules do not penetrate within the actual molecules, but only over the molecular surfaces. We have studied the effect of adsorbed water on the semiconductivity of a solid specimen of methanol-denatured hæmoglobin, allowing three days for equilibrium for each point. The result in Fig. 1 shows that there is a large increase in conductivity which reaches a constant value at about V_m. Thereafter, there is little change until a relative vapour pressure of about 0·9, corresponding to $4V_m$, when the conductivity increases sharply to about 10^{-8} Ω^{-1} cm.$^{-1}$ at 30° C.

Following the result of the previous communication, it is suggested that initially water molecules are held on special sites and donate electrons into the conductivity band of the protein molecule giving an '*N*-type (negative) protein', where a high mobility is expected throughout the crystal[8]. To accord with earlier suggestions[13], and the increase in conductivity, these sites may be CONH groups adjacent to polar side chains. This view differs from that of Baxter[14], who suggested that electrons tunnelled from one adsorbed water molecule to the next. As the physical surface becomes filled up around $4V_m$, we expect proton conduction to set in within the monolayer of adsorbed water, along lines suggested by Riehl[15]. Proton conduction, with 10^{-9} Ω^{-1} cm.$^{-1}$, has been

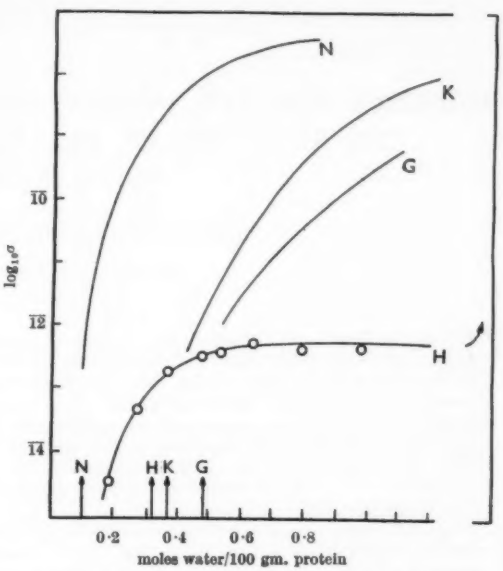

Fig. 1. Specific conductivity σ, Ω^{-1} cm.$^{-1}$ as a function of adsorbed water for nylon at 25° C. (*N*) (ref. 9), keratin at 25° C. (*K*) (ref. 9), gelatin at 32° C. (*G*) (ref. 11) and hæmoglobin (*H*) at 30° C. The arrows mark the values of the Brunauer–Emmett–Teller monolayer adsorption (refs. 11 and 17)

demonstrated for keratin with 15 per cent water[16], about $2{\cdot}5V_m$ [17] (see Fig. 1). Similar 'high' specific conductivities may be calculated for gelatine with $2{\cdot}5V_m$ adsorbed water[18]. It may be that the relatively marked effect of water on the conductivity of these two fibrous proteins, and its even more marked effect on nylon[16,19] point to the operation of yet a third effect due to adsorbed water on flexible chain molecules containing CONH groups, that is, the plasticizing effect of water molecules on chain movement, facilitating transfer of protons between NH and CO groups in different chains, first described by Baker and Yager[19].

D. D. Eley
D. I. Spivey

Chemistry Department,
University of Nottingham.

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EVIDENCE FOR THE INVOLVEMENT OF A TRYPTOPHAN RESIDUE IN THE REACTION OF DI*ISO*PROPYLPHOSPHOROFLUORIDATE WITH α-CHYMOTRYPSIN

By JOHN F. WOOTON and Prof. GEORGE P. HESS

Department of Biochemistry, Cornell University

THE inhibition of chymotrypsin by di*iso*propylphosphorofluoridate was first reported by Jansen, Nutting, Jang and Balls[1]. In this stoichiometric reaction[2,3] an aliphatic hydroxyl group of a serine residue in the active centre of chymotrypsin is implicated[4]. Evidence is presented in this communication which indicates that a tryptophan residue is also involved in the reaction of di*iso*propylphosphorofluoridate with α-chymotrypsin.

The difference spectrum of di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin (three times crystallized α-chymotrypsin, Worthington Biochemicals, Freehold, New Jersey) at *p*H 6·7 is illustrated in Fig. 1*a*. Identical solutions of α-chymotrypsin are added to the sample cell and to the reference cell of a Cary model 14 self-recording spectrophotometer. Approximately three times the stoichiometric amount of di*iso*propylphosphorofluoridate in dilute *iso*propanol is added directly to the sample cell while an equivalent aliquot of dilute *iso*propanol is added to the reference cell. The solutions are scanned 3 min. after addition of di*iso*propylphosphorofluoridate and at intervals up to 30 min. later. No spectral changes are observed on standing, indicating that the reaction is complete in 3 min. at this *p*H, and that the spectral change is due to the addition of di*iso*propylphosphorofluoridate and not to any progressive change of the control solution due to autolysis.

Nitrogen[5] and phosphorus[6] determinations on exhaustively dialysed samples of di*iso*propylphosphoryl α-chymotrypsin prepared using 3 and 10 times the stoichiometric amount of di*iso*propylphosphorofluoridate showed a phosphorus content of 1·16 moles per mole of enzyme, based on a molecular weight of 25,000 and a nitrogen content of 16·5 per cent. This is in good agreement with the previously reported value[2].

The difference spectrum of di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin appears to be due to the specific reaction of an acylating agent with the active site of the enzyme. When α-chymotrypsin denatured by 8 *M* urea or α-chymotrypsinogen are treated with a ten-fold excess of di*iso*propylphosphorofluoridate, and these samples are scanned versus their respective control sample, difference spectra are not observed. The difference spectrum of di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin is essentially the same as the difference spectrum of one of the monoacetyl-α-chymotrypsins[7] versus α-chymotrypsin (Fig. 1*b*). Above *p*H 6·0, where monoacetyl-α-chymotrypsin deacylates, the difference spectrum disappears with time. The rate of change of ΔD 290 appears to follow the known rate of deacylation of monoacetyl-α-chymotrypsin at the same *p*H and temperature.

A series of experiments were carried out in order to differentiate between various types of interactions which could result in the appearance of the difference spectrum of di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin. (1) The relationship between ΔD 290 and concentration of di*iso*propylphosphoryl-α-chymotrypsin follows Beer's law up to a concentration of 1·15 mgm. of di*iso*propylphosphoryl-α-chymotrypsin per ml. (Enzyme concentrations were determined spectrophotometrically at 280 mμ using $E(280, 1 \text{ per cent}) = 20·0$ to relate extinction to enzyme concentration[8].) (2) The difference spectrum is stable over the *p*H range 1–11, both in water and in 8 *M* urea. Large changes in ionic strength do not

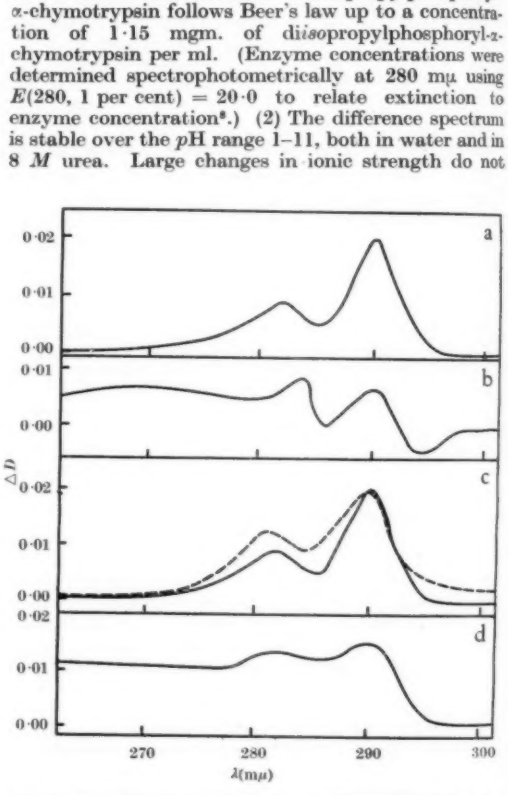

Fig. 1. Ultra-violet difference spectra. *a*, Di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin, enzyme concentration ≈ $3·6 \times 10^{-5}$ *M*, *p*H 6·7, 0·033 *M tris* hydrochloric acid, 0·033 *M* calcium chloride, $1·1 \times 10^{-4}$ *M* di*iso*propylphosphorofluoridate, 25° C.; *b*, monoacetyl-α-chymotrypsin versus deacylated control, enzyme concentration ≈ $2·4 \times 10^{-5}$ *M*, *p*H 6·7, 0·033 *M tris* hydrochloric acid, 0·033 *M* calcium chloride, 25° C. Spectrum obtained 6 sec. after adjusting *p*H of monoacetyl-α-chymotrypsin solution to 6·7; *c*, effect of ionic strength on difference spectrum of di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin, enzyme concentration ≈ $3·6 \times 10^{-5}$ *M*, 25° C. The enzyme samples were dialysed at 4° C. for 72 hr. against several changes of de-ionized water. The ionic strength was adjusted by addition of sodium chloride. ———, $\mu = 0·5$ *M*; – – –, $\mu = 6 \times 10^{-4}$ *M*; *d*, di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin in 8 *M* urea, enzyme concentration ≈ $3·6 \times 10^{-5}$ *M*, *p*H 7·0, $\mu = 0·2$ *M*, 25° C.

appreciably affect either the magnitude or the position of the principal peak of the difference spectrum at 290 mμ (Fig. 1*c*). (3) The difference spectrum of di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin persists after denaturation of the enzymes in 8 *M* urea at *p*H 7·0 for 48 hr. at 37° (Fig. 1*d*). It has been demonstrated[9], using optical rotation as criterion, that both chymotrypsin and chymotrypsinogen are completely denatured in 7 *M* urea at 0° C. after 2½ hr. Di*iso*propylphosphoryl-α-chymotrypsin is more rapidly denatured in urea than chymotrypsinogen[10].

The data presented so far suggest that the spectral shift responsible for the 290-mμ peak is not due to some form of intermolecular interaction, intramolecular hydrogen bonding, a simple dipole–dipole interaction, a change in dielectric constant of the medium surrounding the chromophore, or a charge effect.

The findings are consistent with a strong interaction involving either a tyrosine or a tryptophan residue in di*iso*propylphosphoryl-α-chymotrypsin, since both these residues absorb strongly in the 280–300 mμ region. In order to differentiate between these two amino-acid residues, advantage is taken of differences in their spectral behaviour at high *p*H. The characteristic 282 mμ absorption peak of tryptophyl residues in proteins undergoes only slight shifts with changes in *p*H (ref. 11). The absorption maximum of unionized tyrosyl residues in proteins is approximately at 278 mμ, of ionized tyrosyl residues at 295 mμ. The observable increase in optical density at approximately 295 mμ with ionization of tyrosyl groups makes possible a spectrophotometric titration of these groups[12]. Thus abnormalities in the behaviour of tyrosyl groups in proteins due to interaction with other groups are detectable. To determine whether a tyrosyl group ionizes differently at any *p*H in di*iso*propylphosphoryl-α-chymotrypsin than in α-chymotrypsin, pairs of samples are compared in both aqueous buffer and in 8 *M* urea at *p*H's between 7 and 13.

Table 1. $\triangle D$ 295, DI*iso*PROPYLPHOSPHORYL-α-CHYMOTRYPSIN VERSUS α-CHYMOTRYPSIN

Enzyme concentration, $3{\cdot}6 \times 10^{-5}$ *M*, $\mu = 0{\cdot}2$ *M*, 23°. A Beckman model *G* *p*H meter was used for *p*H determinations

8 *M* urea		Aqueous	
*p*H	$\triangle D$ 295	*p*H	$\triangle D$ 295
7·4	+ 0·002	7·2	+ 0·001
7·8	0·000	7·9	0·000
9·3	+ 0·010	9·3	0·000
9·8	− 0·006	9·8	+ 0·013
12·3	+ 0·004	11·3	+ 0·005
13	+ 0·002	12·6	+ 0·003

As can be seen from Table 1, there is no significant difference between di*iso*propylphosphoryl-α-chymotrypsin and α-chymotrypsin in the ionization behaviour of tyrosine residues. The data suggest that a tryptophan and not a tyrosine residue is modified in di*iso*propylphosphoryl-α-chymotrypsin.

This suggestion is supported by studies of the reaction of N-bromosuccinimide with di*iso*propylphosphoryl-α-chymotrypsin and α-chymotrypsin. According to previous reports the reaction conditions used in these experiments lead to the selective oxidation of the tryptophan residues to the corresponding oxindole[13]. The reaction was carried out in 8 *M* urea after the enzymes were allowed to stand in 9 *M* urea for 24 hr. Under these conditions all tryptophan residues should be equally reactive. It was found, however, that oxidation of tryptophans

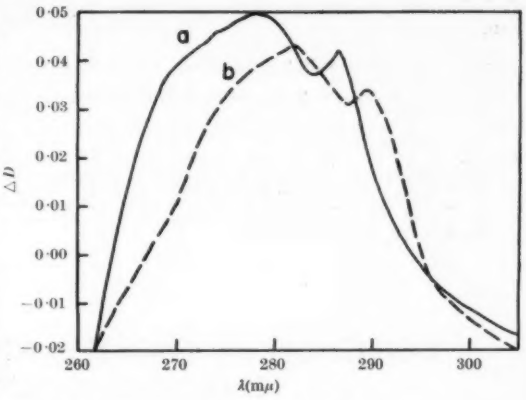

Fig. 2. Ultra-violet difference spectra in 8 *M* urea following reaction of enzyme preparation with N-bromosuccinimide. Enzyme concentration ≈ $3{\cdot}6 \times 10^{-5}$ *M*, *p*H 4·0, 0·07 *M* sodium acetate buffer, 25° C. The sample cell contains an enzyme preparation in which 4·0 moles of tryptophan residues were oxidized by N-bromosuccinimide per mole of enzyme; the reference cell contains an enzyme preparation in which 4·5 moles of tryptophan residues were oxidized per mole of enzyme. Curve *a*, di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin; curve *b*, α-chymotrypsin versus α-chymotrypsin

by N-bromosuccinimide occurs less readily in di*iso*propylphosphoryl-α-chymotrypsin than in α-chymotrypsin. Addition of the same amount of N-bromosuccinimide to identical solutions of α-chymotrypsin and di*iso*propylphosphoryl-α-chymotrypsin leads to the oxidation of 4·5 ± 0·1 residues in α-chymotrypsin and only 4·0 ± 0·1 residues in di*iso*propylphosphoryl-α-chymotrypsin. The difference spectra of N-bromosuccinimide-treated di*iso*propylphosphoryl-α-chymotrypsin versus N-bromosuccinimide-treated α-chymotrypsin, and N-bromosuccinimide-treated α-chymotrypsin versus N-bromosuccinimide-treated α-chymotrypsin are illustrated in Fig. 2. The sample cell contains di*iso*propylphosphoryl-α-chymotrypsin or α-chymotrypsin in which 4·0 moles of tryptophan are oxidized per mole of enzyme; the reference cell contains α-chymotrypsin in which 4·5 moles of tryptophan are oxidized per mole of enzyme. In both cases the difference spectra correspond closely in shape to an absolute spectrum of tryptophan, but they differ both in position and in the relative intensities of the absorption peaks. The data indicate that the reaction of N-bromosuccinimide with di*iso*propylphosphoryl-α-chymotrypsin leads to the accumulation of a reaction product which is not found in the reaction of N-bromosuccinimide with α-chymotrypsin.

The results presented suggest that the 290 mμ absorption peak observed in the difference spectra of monoacetyl-α-chymotrypsin versus α-chymotrypsin, and di*iso*propylphosphoryl-α-chymotrypsin versus α-chymotrypsin, is intimately associated with the specific acylation of the active site of α-chymotrypsin. Since these difference spectra seem identical, it appears that their origin is probably not due to a direct interaction with these substituents, but due to a reversible rearrangement of the active site of the enzyme. The results support the view that this rearrangement involves a strong interaction, resembling a covalent bond, with a tryptophan residue in the enzyme.

These investigations were aided in part by the Office of Naval Research, Department of the Navy

(*NR* 108–417) and by the National Science Foundation.

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DETECTION IN INVERTEBRATES OF INDUCIBLE, REACTIVE MATERIALS RESEMBLING ANTIBODY

By JOHN H. PHILLIPS and BENJAMIN J. YARDLEY
Department of Bacteriology, University of California, Berkeley, California

THOSE reactive substances that are produced by vertebrates in response to foreign materials, and to which the term 'antibody' is generally applied, constitute a collection of proteins of somewhat varied properties. The outstanding common characteristic of these proteins is their reaction with the inducing antigen, although the occurrence of this reaction may be manifested in a number of different ways. Apart from assay methods which involve biological response, for example, toxin neutralization, sensitization, etc., quantitative detection is generally dependent on either the production of a change in state of one or both of the reactants, such as precipitation and agglutination, or the consumption of a third measurable component by the antigen antibody reaction as in complement fixation.

The choice of a method of detection for studies on invertebrates might therefore be made from any of the above procedures. However, there are certain difficulties inherent in each of these approaches. Deviations in behaviour sufficient to be scored as biological responses are not readily judged in such animals as sea anemones. Precipitation and agglutination may be dependent on rather unpredictable conditions in the reaction mixture, for example, the concentration of electrolyte or protein. There is variation in the ability of antibodies from various animals to fix complements of heterologous species.

The methods described here were developed in an attempt to circumvent some of these difficulties. These procedures allow the rapid detection of antibody formation in a variety of invertebrates. In essence, we aim at the detection of any combination between induced substances in the invertebrate and the inducing agent. Such combination is detected through the use of a standardized rabbit antiserum prepared against the inducing antigen. Measured amounts of rabbit antiserum and antigen are added to invertebrate preparations. Any deviation from the expected amount of precipitate due to the combination of rabbit antibody and antigen is taken as presumptive evidence of a combining substance present in the extract of the invertebrate. Further evidence is obtained by demonstrating a relationship between the injection of antigen into the animal and the subsequent increase in activity of extracts. Finally, a certain degree of specificity must be demonstrated by showing a difference in the effect of the invertebrate extracts on a precipitating system in which a different antigen and rabbit antibody are used.

Investigations on the anemone, *Anthopleura elegantissima*, are reported here. Individual animals were given an injection of 1 mgm. of Armour bovine serum albumin fraction V, contained in 0·1 ml. of artificial sea water[1]. In each case an attempt was made to introduce the antigen into the mesoglœa, but success or failure was difficult to judge. Most of the animals were placed in a 30-gallon aquarium in which sea water was maintained at 13° C. At the same time, six anemones were placed in small, individual jars containing 50 ml. of artificial sea water and maintained at the same temperature. Since cœlenterates are nonvascular animals, extracts were prepared from the whole animals as follows: 5 anemones were removed from the aquarium at desired intervals of time and individually macerated in a Waring blender with artificial sea water containing 1/10,000 merthiolate. The volume of sea water employed was 100 ml., including the volume of the anemone. The water from the six small jars containing individual anemones was collected after the same time intervals and replaced by fresh artificial sea water. The extracts and sea water samples were centrifuged at 15,000 r.p.m. in a high-speed 'Serval' for half an hour at 5° C., and the supernatant filtered through Whatman No. 1 filter paper. The filtrate was then tested for antibody activity.

Two methods of testing for activity have been employed. The scheme of the first is shown in Table 1. Each of the mixtures was set up in duplicate. Incubation was at 5° C. for 24 hr. The mixtures were then centrifuged at 5° C. for 15 min. at 2,000 r.p.m., and the precipitates washed twice with artificial sea water at 5° C. The precipitates were dissolved in 0·1 *N* sodium hydroxide and the amount of protein measured by the Lowry method[2], using a calibration curve prepared using bovine serum albumin. The method gave a reproducibility of $\pm 0{\cdot}008$ mgm. protein.

The second test method involved a similar scheme with the use of 2·0 ml. of test material and one-half the amounts of bovine serum albumin and rabbit

Table 1

Mixture	Test material*	Bovine serum albumin	Rabbit anti-bovine serum albumin diluted 1/5	Rabbit normal serum diluted 1/5	Artificial sea water
	ml.		ml.	ml.	ml.
1		0·5 ml. (0·0155 mgm.)	0·5		4·0
2		0·5 ml. (0·0155 mgm.)			4·5
3			0·5		4·5
4				0·5	4·5
5	4·0			0·5	0·5
6	4·0				1·0
7	4·0	0·5 ml. (0·0031 mgm.)			0·5
8	4·0		0·5		0·5
9	4·0	0·5 ml. (0·0155 mgm.)	0·5		

* Sea anemone extract or ambient sea water from injected anemones.

anti-bovine serum albumin in a total volume of 3·0 ml. The reactants were placed in 10 mm. cuvettes in the Beckman *D.U.* spectrophotometer, and the turbidity was measured at intervals for 65 min. at a wave-length of 340 mμ using a slit-width of 0·218 mm. and a blank containing rabbit antiserum and artificial sea water. Temperature was maintained at 25° C. The method of measurement is essentially the same as that employed by Najjar *et al.*[3]. The optical density is observed to increase sharply during the first 30 min. of observation. By 60 min. the optical density becomes quite constant and there is only a small increase in the following 30 min.

Extracts which tended to darken on standing could be cleared by treatment with 'Nuchar *C*–190–*N*' activated charcoal (Industrial Chemical Sales Division, West Virginia Pulp Paper Co.). The treatment with 'Nuchar' could not be used prior to the determination of residual bovine serum albumin as this protein is adsorbed during the removal of the interfering brown pigment. No detectable change in the antibody-like activity was produced by the use of the small amounts of 'Nuchar' employed.

In both test methods, the results were evaluated by comparison with a standard curve prepared using rabbit antiserum and bovine serum albumin diluted with artificial sea water. The relationship between the two methods of measurement is shown in Fig. 1.

The levels of bovine serum albumin and anti-bovine serum albumin used in all tests lie in the region of great excess of antibody. The reproducibility of the turbidimetric method was found to be within 0·004 optical density. It was observed that the values obtained by either method of measurement could be evaluated in terms of the other. The use of both methods of assay allows observation of the course of the reaction and a check of the final result.

Mixtures 1–4 acted as controls of the rabbit precipitating-system and were included in each group of tests. No significant difference in the blank values, mixtures 5 and 6, was observed in any experiment. These two mixtures acted as controls of the anemone extract. There was no indication of precipitation in mixture 7, demonstrating a lack of anemone antibody capable of precipitation under the conditions of the tests.

The precipitate in mixture 8 represented residual bovine serum albumin from the injection. The disappearance of injected bovine serum albumin from the animal, as indicated by the tests of the extracts,

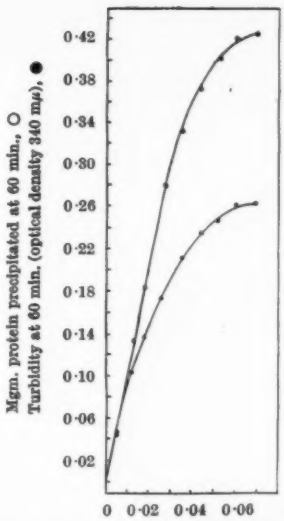
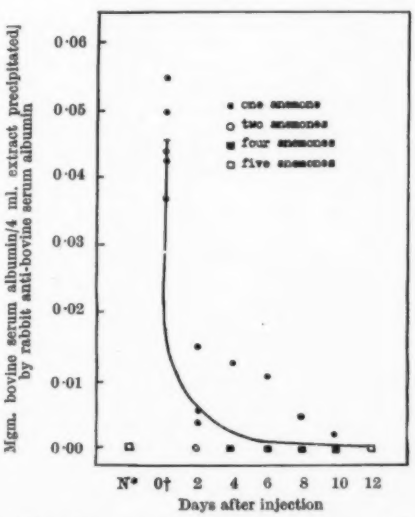


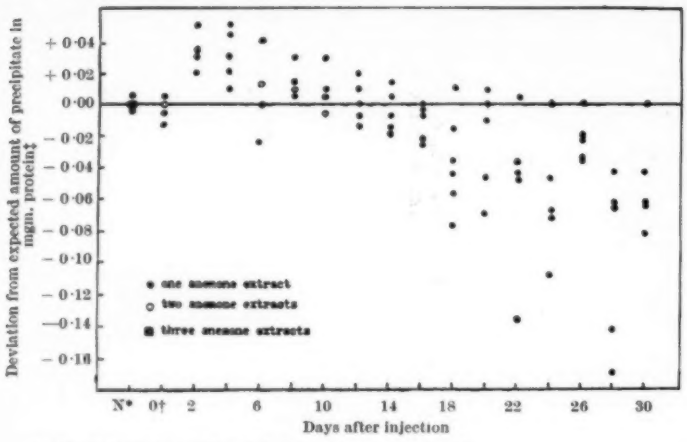
Fig. 1

follows the course indicated in Fig. 2, which includes a plot of the average amount of residual bovine serum albumin per 4 ml. of extract. From day 12 onwards, the amount of bovine serum albumin remaining was below the level of detection in all anemones. (The level of detection was 2 μgm. bovine serum albumin.) In no case was bovine serum albumin detected in the sea water surrounding injected anemones. If leakage of this antigen from the animals were responsible for the observed decrease, the albumin released would have been detected, because it would have been contained in half the volume used for preparation of the extracts. It may there-



* Normal anemones not injected with bovine serum albumin.
† Anemones immediately after injection of bovine serum albumin.

Fig. 2



* Normal anemones not injected with bovine serum albumin.
† Anemones immediately after injection of bovine serum albumin.
‡ All negative deviations have been corrected to deviation per 5 mgm. of extract protein.

Fig. 3

fore be concluded that the disappearance of the bulk of the injected antigen represents breakdown within the anemone rather than excretion. Examination of extracts over a period of time indicated that there was no destruction of bovine serum albumin due to proteolytic enzymes once the extract was prepared. Since observations on intracellular digestion in cœlenterates indicate a distinctly acid condition in vacuoles containing materials undergoing hydrolysis, the observed absence of breakdown may be due to the somewhat alkaline reaction of the extracts due to the use of sea water as extractant[4].

Fig. 3 shows the deviations from the amount of precipitate expected in mixture 9 observed with extracts prepared from anemones. The expected amount of precipitate varied between 0·11 and 0·13 mgm. of protein, depending on the lot of rabbit antibody and bovine serum albumin used.

All deviations have been corrected for residual bovine serum albumin due to the injection. Since the anemones varied in volume from 2 to 20 ml., the negative deviations have been adjusted to give values per 5 mgm. of extract protein. The positive deviations could not be adjusted in a similar manner for reasons that will be discussed below. The maximum deviations actually measured were +0·05 and −0·128 mgm. protein.

Twenty-four hr. after the injection of bovine serum albumin, extracts appeared to add protein to the rabbit antibody-antigen precipitate. This additive effect gradually disappeared and was replaced by an inhibitory one. At about the eighteenth day after injection, strongly inhibitory extracts were found. Most of the extracts prepared from anemones throughout the remainder of the period of observation showed negative deviations. In an attempt to find a quantitative relationship between the kind of deviation produced and the concentration of anemone material, various dilutions of a strongly inhibitory extract were tested using a constant amount of bovine serum albumin and rabbit antibody. There was a decrease in the negative deviation by 0·0055 mgm. protein for each two-fold decrease in concentration of the anemone material, until the extract had been diluted to the point where the addition of protein could be observed. The additive effect disappeared on further dilution. Therefore, it appears that the inhibitory substance in anemone extracts is the same substance that is responsible for the positive deviations observed in anemones harvested soon after the injection of bovine serum albumin. The observed relationship between concentration of extract and magnitude of deviation was used in adjusting the negative deviations shown in Fig. 3.

The course of antibody synthesis as indicated by the examination of groups of anemones at intervals of time after the immunizing dose could be duplicated by testing one anemone repeatedly. Before injection, a small piece of tissue was cut from an anemone using a 5 mm. cork borer, and the antibody was extracted in the following manner. The tissue was ground in a chilled 12-ml. conical glass centrifuge tube using an etched glass stirring rod. Lipid and pigments were extracted, using a mixture of 3 parts of 95 per cent ethanol and 1 part of distilled water, previously chilled to −5° C. Two 12-ml. volumes of the ethanol solution were employed and the sediment collected by centrifugation at 2,000 r.p.m. for 5 min. after each extraction. The sediment was washed twice with chilled distilled water, allowed to drain thoroughly and finally extracted with 6 ml. of artificial sea water containing merthiolate. No activity could be observed when the extract from a non-immunized anemone was tested using the bovine serum albumin precipitating system. Bovine serum albumin was injected as described above. At daily intervals, pieces of tissue were removed and extracted. The consecutive extracts revealed again the additive and inhibitory effects described above; inhibitory extracts also showed the additive behaviour on dilution.

The specificity of the anemone material has been examined by the use of a precipitating system consisting of human serum albumin and rabbit anti-human serum albumin. Extracts from anemones immunized with bovine serum albumin did not affect the human serum albumin precipitating system. Extracts from anemones similarly immunized with human serum albumin produce no deviation of the bovine serum albumin precipitating system, but show additive and inhibitory effects when tested with the human serum albumin system.

In summary, there are indications that an inducible reactive substance may be elicited in anemones. This substance appears to have a degree of specificity comparable to rabbit antibody. The additive property of anemone antibody shows some interesting characteristics which are now being investigated.

This work has been supported by grants from the U.S. Public Health Service and the National Science Foundation.

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LETTERS TO THE EDITORS

RADIOPHYSICS

New Limits to the Diameters of Some Radio Sources

THE investigations at Jodrell Bank of the angular diameters of extra-terrestrial radio sources have now reached the stage where interferometric observations are being made with a base-line of 32,000 wave-lengths. The interferometer operates at a wave-length of 1·89 m., and uses the 250-ft. steerable paraboloid in conjunction with auxiliary aerials which are now placed about 40 miles to the west of Jodrell Bank near the Welsh coast. This is the longest base-line to be used so far for the observation of radio sources, and it is possible with this instrument to set new limits to the diameter of some radio sources which are significantly smaller than those previously reported[1].

A preliminary experiment is being carried out to investigate the fringe visibility of those sources which produced a measurable fringe when observed at the last base-line used, which was 9,700 wave-lengths. So far, 91 sources have been studied, and of these 38 have produced a measurable fringe. These sources lie in the area between right ascensions 16 hr. and 6 hr., and between declinations 58° and −21°; they have flux densities in the range $8{\cdot}5\text{–}85 \times 10^{-26}$ W.m.$^{-2}$ (c./s.)$^{-1}$. Each source has been observed at least twice and Fig. 1 is a facsimile of one of the records of the transit of source 3*C* 147 (*R.A.* 05h. 38m., Dec. 49° 51′, flux density 63×10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$).

As this is the longest base-line so far used there is no standard radio source of known diameter which can be used to calibrate the observations. The calibration must therefore rely on absolute measurements of fringe amplitude or comparisons with previous observations of the signal to noise ratio of the intense sources. Fig. 2 is a histogram of the results for the 38 sources which produced a measurable fringe. The abscissa is the ratio of the observed fringe amplitude on the recorder chart, corrected only for the polar diagram of the auxiliary aerial, to the flux density listed in the 3*C* catalogue[2]. This is an arbitrary scale dependent on the gain setting and sensitivity of the interferometer, but it is proportional to fringe visibility. A scale of absolute fringe visibility has been estimated from a comparison of the signal to noise ratio observed for three intense sources (3*C* 48, 147 and 459) with that observed at shorter base-lines, and this has been used to set limits to the diameters of the sources. The lower scale on Fig. 2 shows the estimated upper limits to the source diameters. It has been assumed that the sources are simple circular disks with a gaussian distribution of surface brightness; the scale shows the diameters between the points of half-brightness.

These observations will, of course, be affected by confusion and noise. However, the effective solid angle in which confusing sources can be observed is limited by various factors to less than half a square degree. It is estimated that, with this very small area, the error in fringe amplitude due to confusing sources (even if the confusing sources are unresolved) should not exceed 2×10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$ in 90 per cent of these observations.

These preliminary observations show that out of 91 sources at least seven have major features smaller than 3 seconds of arc, and that these sources have an extremely high surface brightness which is comparable with that of the intense radio source in Cygnus, I.A.U. 19*N*4*A*. The source I.A.U. 14*N*5*A* has recently been identified[3] with an object having a recessional velocity approaching half the speed of light. The

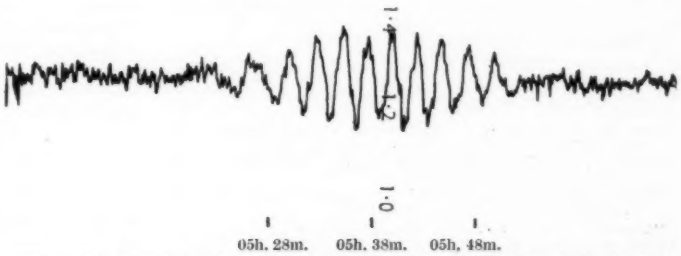

Fig. 1. Record of the transit of the source at *R.A.* 05h. 38m. obtained on September 19, 1960

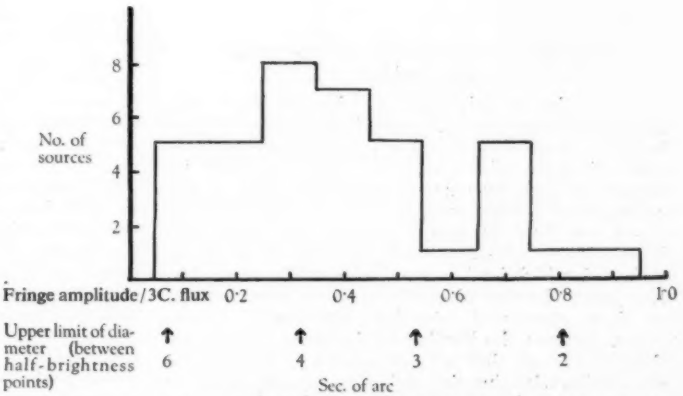

Fig. 2. Histogram of the normalized fringe amplitudes for the 38 sources which produced measurable fringes at 32,000 wave-lengths resolution. The scale of diameters has been estimated from a comparison of signal to noise ratio at this and shorter base-lines

diameter of this radio source has been estimated, from observations at 9,700 and 32,000 wave-lengths, as 4½ sec. of arc in position angle 090°. This identification supports the view that these sources of small angular diameter and high surface temperature comparable to that of Cygnus *A* are objects at such great distances that cosmological effects should be significant.

It has been pointed out by Hoyle[4] and by Davidson[5] that there is a theoretical lower limit to the observable diameter of a radio source which is dependent on the particular cosmology considered. They have shown that, for sources similar to Cygnus *A*, this lower limit is 4 sec. of arc for a steady-state universe and 15 sec. of arc for an Einstein–de Sitter universe. The results in Fig. 2 show that a significant fraction of radio sources have an angular size less than the values predicted for either a steady state or an Einstein–de Sitter universe, but in making this comparison it must be remembered that the source Cygnus *A* is asymmetrical[6,7] with a ratio of major to minor axes greater than 4. All the measurements reported here refer to position angle 090°, and do not take account of any asymmetry in the sources.

We wish to thank Prof. A. C. B. Lovell for making available the facilities of Jodrell Bank, and both him and Prof. R. Hanbury Brown for their continued interest and advice during the course of this work. One of us (B. R.) is indebted to the Leverhulme Trust for a research fellowship.

L. R. Allen
H. P. Palmer
B. Rowson

Nuffield Radio Astronomy Laboratories,
Jodrell Bank.

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Propagation of Whistlers to Polar Latitudes

Within the past three years, whistlers have been heard consistently at a number of stations located on the polar side of the auroral zones. Certain characteristics of polar whistlers have been reported by Martin[1] for Scott Base (geomagnetic latitude 79·3° S.), by Ungstrup[2] for Godhavn (79·8° N.) and by Helliwell *et al.*[3] for Byrd Station (70·6° S.). In each case similarities to whistlers received at lower latitudes are noted, which lend support to Martin's suggestion that polar whistlers originate in lower latitudes and are then propagated into the polar regions by reflexion between the Earth and the lower ionosphere. The purpose of this communication is to describe observations showing that lightning sources in middle latitudes can give rise to whistlers heard at polar latitudes.

Simultaneous observations of whistlers have been made regularly since June 1958 at Scott Base, Ross Dependency, Antarctica, and at Dunedin and Wellington, New Zealand, situated 3,600 km. and 4,100 km. north of Scott Base, respectively. The geomagnetic latitudes of Dunedin and Wellington are 50·8° S. and 45·4° S. respectively. The times of occurrence of whistlers at these stations are normally reported to the nearest second.

A statistically significant association has been found between the occurrence of whistlers at Scott Base and of those reported from the stations in New Zealand. For example, for the first six months of 1960, during a total of 941 two-minute recording periods, 2,946 whistlers were heard at Wellington, while 1,548 were heard at Scott Base. Of these, 489 were found to be coincident. The number of coincidences expected to occur by chance is 48·5; thus there were 10·1 times more coincident whistlers than would be expected by chance. A similar analysis on a more limited series of results from Dunedin and Scott Base gave a ratio of actual coincidences to chance coincidences of similar magnitude (12·2). Statistical (chi-squared) tests applied to these results show that the correlation of occurrences of whistlers at these stations is highly significant.

Attempts have been made to examine the dispersion characteristics of coincident whistlers in order to determine whether these are identical at the three stations. A difficulty arises in obtaining readable spectrograms in that, when the field-strength of a whistler is strong at the New Zealand stations, it tends to be very weak at Scott Base, and vice versa. However, I have succeeded in obtaining readable spectrograms of long whistlers occurring at all three stations at 08h. 36m. 11s. U.T. on April 7, 1959. (The pattern of atmospherics occurring during a few seconds on either side of this time was used to obtain an accurate comparison of times.) At each station the whistler, which was swishy in character, was preceded by a loud atmospheric of the type classified as a 'bonk' by Morgan and Dinger[4]; on analysis, this bonk was identified as the initiating atmospheric. It had an unusually complex structure, indicating that multiple lightning discharges had taken place at 20–30 millisec. intervals during a total time of 0·19 sec.

The dispersion analysis was carried out using an 11-channel comb-filter analyser, the filter frequencies f being arranged to give equal increments of $f^{-1/2}$ between channels, so that dispersion curves obeying the Eckersley–Storey approximation[5] would be straight lines. The times of first and last observable response for each channel (starting and finishing times) are given in Fig. 1*a* and Fig. 1*b* respectively, time being measured from the commencement of the initiating bonk. The dispersion lines shown in Fig. 1*a* refer to the commencement of the bonk, and those in Fig. 1*b* refer to its end. Although the starting times at these three stations do not agree well, nevertheless, apart from the two exceptions, discussed below, the agreement of the finishing times is within the accuracy of determination of times from the spectrograms (normally about ± 0·02 sec.). These points closely describe a whistler component of dispersion 164 sec.$^{1/2}$, arising from the last component in the multiple lightning discharge.

An analysis of the wave-forms of the initiating bonk, as recorded at the three stations, suggested that the lightning-source was 1,100 ± 200 km. distant

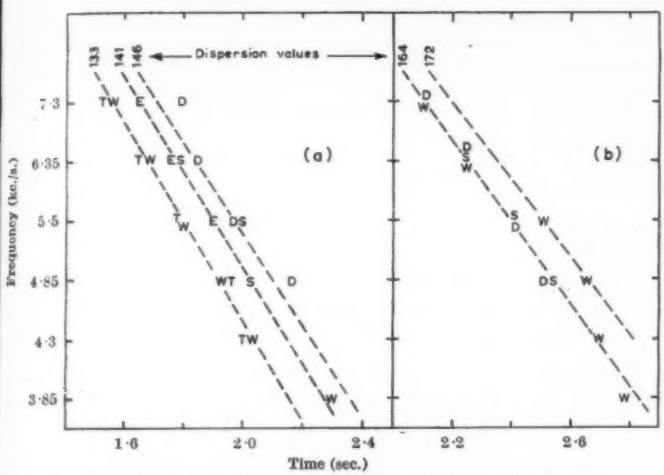

Fig. 1. Dispersion characteristics of whistlers apparently arising from the same source, as recorded at Scott Base (*S*), Dunedin (*D*), Wellington (*W*), Stanford (*T*) and Seattle (*E*). The northern hemisphere times have been adjusted as described in the text. (*a*) Starting times; (*b*) finishing times

from both Wellington and Dunedin, and 4,350 ± 300 km. from Scott Base, that is, in the vicinity of lat. 38° S., long. 163° E., in the middle of the Tasman Sea. The New Zealand Meteorological Service has kindly supplied the information that a vigorous depression had its centre at lat. 39° S., long. 163° E., at 0600 U.T. on the day in question. This centre had moved to lat. 40° S., long. 165° E., by 1200 U.T. Thunderstorms and cumulo-nimbus clouds reaching altitudes of 20,000 ft. were reported by ships and aircraft in the area at this time. The synoptic weather charts indicate that this was the only likely thunderstorm area for some thousands of miles around. Therefore it appears to be reasonably certain that this region, at a geomagnetic latitude of about 43° S., gave rise to the whistler heard at Scott Base.

Sonagrams of short whistlers recorded at Stanford and Seattle, U.S.A., which apparently arose from the same source, have been kindly supplied by Mr. L. H. Martin, of Stanford University. The geomagnetic latitudes of these stations are 43·7° N. and 53·6° N. respectively. Both sonagrams show multiple component structure. The first components are well defined, especially on the Stanford record, but in both cases there is no clearly distinguishable last component because of high noise-level from local atmospherics. The initiating atmospheric was readily identified with that found on the southern hemisphere spectrograms. A time correction of 0·04 sec. was made, to allow for the time of propagation of the atmospheric from its source to the west coast of the United States. The times thus corrected were then doubled to permit direct comparison with the southern hemisphere data. This is shown in Fig. 1*a*. It will be seen that the Stanford starting times agree well with the Wellington times for frequencies between 4·3 and 7·3 kc./s., and describe a whistler component of dispersion 133 sec.$^{1/2}$ arising from the initial lightning discharge. On the other hand, the Seattle starting times are consistent with two of the three Scott Base starting times and the 3·85 kc./s. response at Wellington, indicating a whistler component of dispersion 141 sec.$^{1/2}$. The lowest-latitude stations in each hemisphere (Stanford and Wellington) have thus recorded an earlier whistler component than the higher-latitude stations. The explanation of this may involve consideration of the terminal latitudes of the paths along which the various components were propagated through the outer ionosphere. The anomalous lateness of starting times at Dunedin is attributed to the exceptionally high noise-level there at that time.

In view of the way in which results from the various stations in both hemispheres correlate, and also in view of the multiple structure revealed by the Stanford and Seattle sonagrams, it seems reasonable to attribute the later finishing times at Wellington for frequencies of 4·85 and 5·5 kc./s. to the reception there of an additional component which was not recorded at Dunedin or Scott Base because of higher noise-levels at these two stations.

An extension of the dispersion analysis of the Wellington whistler showed no sign of a nose frequency up to 14·6 kc./s., the highest frequency at which there was a useful response. On the dipole representation of the geomagnetic field, this implies that the path of highest latitude for this whistler must have terminated below 60° geomagnetic latitude; if the nose frequency were one-half of the minimum gyrofrequency over the path, the maximum terminal latitude would be less than 55°. Since the nose frequency is likely to be at least 20 kc./s., the maximum terminal latitude is in fact likely to be less than 53°. Following an alternative line of reasoning, that of comparing the measured dispersion values with an empirical curve of the variation of dispersion with latitude given by Allcock[6], a maximum terminal latitude of about 51° is inferred. The general agreement between these two estimates of the maximum terminal latitude makes it reasonably certain that the whistler under consideration not only arose from a source in middle latitudes, but also was indeed a true middle-latitude whistler, being propagated through the outer ionosphere along paths which terminated in middle latitudes. Reception of this whistler at the polar station of Scott Base would therefore imply a final stage of propagation underneath the ionosphere for more than 2,800 km.

This work forms part of the radio research programme of the Dominion Physical Laboratory. I am grateful to Prof. R. A. Helliwell of Stanford University for his assistance in providing equipment, and to the Antarctic Division, Department of Scientific and Industrial Research, New Zealand, for operating the Scott Base whistler equipment.

G. McK. Allcock

Dominion Physical Laboratory,
Lower Hutt,
New Zealand.

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PHYSICS

Simultaneous Application of Townsend and Streamer Theory

Recently conductivity current measurements up to the sparking potential have been carried out in hydrocarbon gases which can be interpreted if it is assumed that Townsend and streamer processes act simultaneously.

A sample measurement for *iso*pentane is shown in Fig. 1. At $E/p = 300$ V. cm^{-1}/mm. mercury, $p = 3$ mm. mercury, the d.c. ionization current was measured, by successive reduction of i_0, over more than eight decades of amplification, without any sign of a deviation from the normal α slope, up to the sparking voltage of 990 V. which occurred at $d = 1{\cdot}1$ cm. The last slope was obtained with 10 electrons leaving the cathode per second and this type of measurement has often been repeated and confirmed.

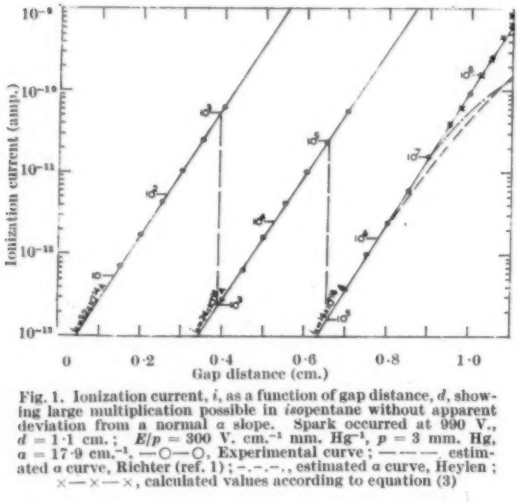

Fig. 1. Ionization current, i, as a function of gap distance, d, showing large multiplication possible in *iso*pentane without apparent deviation from a normal α slope. Spark occurred at 990 V., $d = 1{\cdot}1$ cm.; $E/p = 300$ V. cm.$^{-1}$ mm. Hg^{-1}, $p = 3$ mm. Hg, $\alpha = 17{\cdot}9$ cm.$^{-1}$, —○—○, Experimental curve; — — —, estimated α curve, Richter (ref. 1); –·–·–, estimated α curve, Heylen; ×—×—×, calculated values according to equation (3)

By monitoring the pulse shape of the ionic component of individual avalanches, it has been observed[1] that the number of charge carriers (n) increases less than $\exp(\alpha x)$ if n surpasses $N = 10^6$. The space charge field of the positive ions is held responsible for the reduction of the ionization of the advancing electrons and is comparable to the applied field; we are thus entering the streamer regime. It was found (for ether, a gas exhibiting an extremely low γ coefficient) that for $n \geqslant N$, Townsend's α coefficient could be expressed as:

$$\alpha(n) = \alpha\,(1 - B \ln n/N) \qquad (1)$$

in which $B = 0{\cdot}065$. From this, the charge multiplication factor was evaluated as:

$$x - x_N = \frac{1}{\alpha}\left\{\frac{1}{1 - B \ln n/N} - \frac{1}{B}\ln(1 - B \ln n/N) - 1\right\} \qquad (2)$$

in which $N = \exp(\alpha x_N)$. The reduction from the normal α slope, according to equation (2), is shown in Fig. 1 (dashed line), and does not agree with the observed data. The discrepancy can be cleared up if it is assumed that Townsend's secondary mechanism is operating simultaneously with the streamer inducing space charge reduction of Townsend's primary process. This can be taken into account by using the generalized Townsend equation:

$$i_x = i_0 \frac{\exp(\int_0^x \alpha dx)}{1 - \gamma\,(\exp(\int_0^x \alpha dx) - 1)} \qquad (3)$$

in which $\exp(\int_0^x \alpha x d)$ can be found from Fig. 1 (dashed line). An estimate of γ can be obtained by making equation (3) fit the experimental curve. Thus, for $\gamma = 4{\cdot}3 \times 10^{-6}$, a normal α slope would be recorded up to $d = 0{\cdot}9$; for longer gaps, an upcurving would occur and the Townsend criterion for breakdown would be reached at $d = 0{\cdot}98$, which is not in agreement with experiment.

From previous work[2] it has been surmized that for normal hydrocarbons, little space charge distortion occurs until $n \geqslant N = 10^7$, and from Richter's data[1] an equally good fit to $\alpha(n)$ can be obtained for $B = 0{\cdot}2$. Substitution of this value into equation (2) yields the curve as shown in Fig. 1 (dot-dashed line). Combining this with equation (3) yields $\gamma = 10^{-8}$; the calculated values are shown (×). It is observed that no apparent deviation from a normal α slope occurs to within 5 per cent of the sparking distance, at which a faster than usual Townsend upcurving takes place.

The award of an Imperial Chemical Industries Fellowship by the University of London is gratefully acknowledged, and also a grant by the Central Research Fund. Thanks are due to Prof. M. W. Humphrey Davies for facilities provided.

A. E. D. Heylen

Electrical Engineering Department,
Queen Mary College,
London.

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The Nitrous Oxide Dosimeter

Nitrous oxide has been proposed[1] as a suitable material for the dosimetry of ionizing radiations over wide temperature- and pressure-ranges and with a wide variety of types of radiation. The basis for the yields quoted in ref. 1 was the measurement of radiation flux using ionization chambers or ferrous sulphate solutions. We have investigated the radiolysis of nitrous oxide and have compared the results of absolute measurements based on tritium as an internal source with measurements based on ferrous or ceric sulphate dosimetry, using cobalt-60 γ-rays or 4-MeV. X-rays as external sources.

It has been found that good agreement between the two methods is obtained provided the irradiations are done in vessels with internal diameters greater than about 15–20 mm. As the internal diameter decreases, the yields of all products, based on ferrous dosimetry, increase in a manner reminiscent of the increase in $G(Fe^{3+})$ from the ferrous sulphate dosimeter in vessels of a few millimetres diameter[2,3].

The products measured were nitrogen, oxygen and the oxides of nitrogen ($NO + NO_2$). Previous workers[1,4] have used liquid nitrogen to separate the volatile and non-volatile constituents of the irradiated gas, but since in this technique reactions occur in the traps which can cause serious errors in the yields of oxygen and oxides, in this work the constituents were not separated. Gas chromatography was used for nitrogen and oxygen analyses, and the oxides of nitrogen were determined spectrophotometrically as nitrite.

$G(N_2)$ was $11{\cdot}0 \pm 0{\cdot}4$ at ambient temperatures and is the average from all the types of radiation mentioned, weighted in favour of the tritium irradiations (for which $G(N_2)$ was $11{\cdot}2 \pm 0{\cdot}2$ and $11{\cdot}3 \pm 0{\cdot}5$ for two experiments).

The most reliable results are likely to be obtained if nitrogen alone is measured, since it is unaffected by trap reactions or other analytical complications, and its yield is also unaffected by foreign gases such as hydrogen or methane, both of which markedly reduce the yields of oxygen and oxides of nitrogen, or by traces of water, which affects the relative proportions of oxygen, nitric oxide and nitrogen dioxide.

A more detailed account of this work will appear elsewhere.

R. W. Hummel
J. A. Hearne

Wantage Research Laboratory,
Atomic Energy Research Establishment,
Wantage,
Berks.

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The Wake of a Moving Drop

Two-dimensional flow behind bluff symmetrical obstacles has received the attention of fluid dynamicists for more than half a century. Considerations of the Kármán vortex-street afford an excellent example of the agreement between theoretical and experimental results. Three-dimensional wakes have been investigated intensively, but are less tractable as they do not exhibit the same uniformity or periodicity as the two-dimensional vortex-trails.

Practical considerations have been an important factor in confining most investigations to phenomena associated with the relative motion between a fluid and a rigid body. More recently, meteorological implications have prompted investigations into the mechanics of liquid drops falling through a quiescent gaseous medium such as air. Many engineering processes involving liquid–liquid phases secure contact through the dispersion of one phase as drops moving through a second liquid.

Telford, Thorndike and Bowen[1] described an experiment in which they determined the collision efficiency between equal-size drops about 150μ in diameter. Schotland[2] modelled the trajectories of cloud droplets of equal size by allowing steel spheres to fall through a sugar solution. The collision efficiencies obtained by these investigators support a wake collision process that may be important in the early stages of cloud drop growth studies. Studies of collisions between larger drops suggest a wake process is operative that may be of some importance in determining the drop-size distribution observed in natural rain. Collisions between equal size drops, moving at the same terminal velocities, suggest a pressure gradient behind the leading drop which accelerates the trailing drop into contact. The time displacement relationships of a drop moving through a medium are directly dependent on the drag force, which in turn depends on the nature of the wake.

Liquid–liquid systems were used in the experiments described in this communication. The wakes of single drops of various organic liquids, moving through water at terminal velocity, were observed and recorded photographically. The wake pattern was rendered visible as a water-soluble dye was scrubbed from the moving drop.

Fig. 1 shows the wake pattern behind a drop of carbon tetrachloride (equivalent diameter 0·24 cm.) moving through quiescent water at 25° C. with a Reynolds number of 360. Fig. 2 shows the wake of the same drop after its energy has been dissipated, but before its identity has been lost through diffusion processes. There is a time-interval of 10 sec. between the two exposures.

The wake behind a drop moving through a liquid phase with Reynolds numbers up to some value, limited by drop oscillations, is characterized by two rows of vortex rings. These rings all move at the same acute forward angle with the line of fall of the drop. The almost perfect symmetry is evident in the wakes of all drops, regardless of the deviation from sphericity, in which there are no local oscillations. Larger oscillating drops result in the production of more energetic rings, but lack the symmetry of the smaller

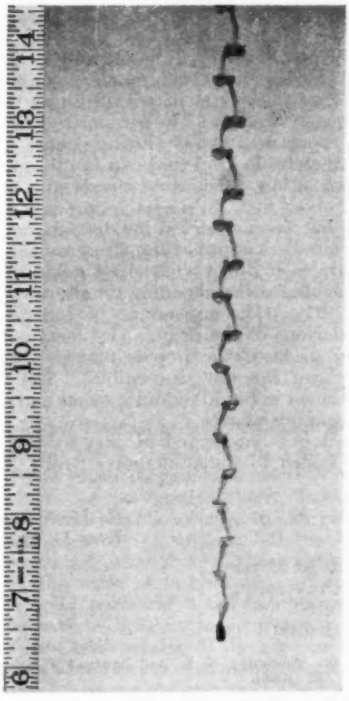

Fig. 1. The wake behind a carbon tetrachloride drop falling through water at terminal velocity. Reynolds number 360. Photograph taken at an angle of 45° with the plane of the wake

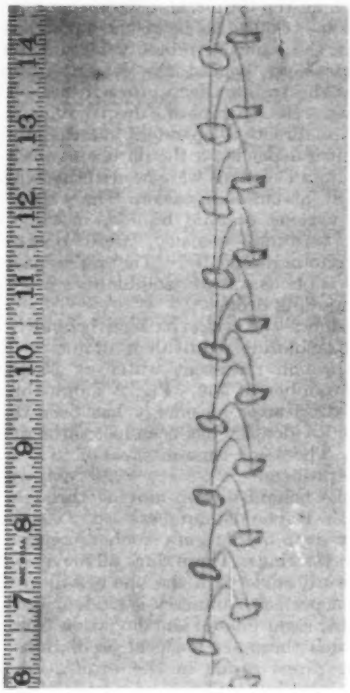

Fig. 2. The wake behind the same drop 10 sec. after the drop has passed

drops. Hu and Kinter[3], Johnson and Braida[4], and Licht and Narasimhamurty[5] report results on drop diameter–drop velocity relationships for carbon tetrachloride–water systems. These reports indicate that 0·32 cm. represents about the maximum size of a carbon tetrachloride drop that can fall through water without oscillation. This corresponds to a Reynolds number of about 900. In these experiments, the wake symmetry has been observed for drop-diameters and Reynolds numbers approaching these values.

Disturbances in the vortex sheet behind the drop are responsible for the shedding on alternate sides of the drop. The wake appears to be independent of gross oscillations of the drop. Any local oscillations or zig-zag motions of the drop as it passes through the disperse phase are not discernible. The work is being continued in liquid–liquid systems and extended to liquid–gaseous phases.

This work was supported in part by the Defence Research Board of Canada, under Grant No. *DRB* 9550–04.

R. H. Magarvey
Roy L. Bishop

Department of Physics,
Acadia University,
Wolfville,
Nova Scotia.

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CHEMISTRY

Organic Ion Flotation

In an earlier communication[1], a procedure for concentrating inorganic ions is outlined. It has been found now that it is possible to concentrate organic ions from aqueous solution by adding a surfactant collector, of opposite charge, suitably prepared to ensure the absence of micelles, and then, by the introduction of fine bubbles, floating the insoluble product to the surface where it collects in the froth, or preferably as a scum. The principle is the same as for inorganic ion flotation, but there is one important distinction. In inorganic ion flotation, because the ions are often polyvalent, the product between the ion and the collector having more than one hydrophobic centre, is highly insoluble and usually forms a scum very quickly. But organic ions are often univalent, and therefore the product, though still insoluble, sheds water less readily, and there is more tendency to form a stable froth. This can, however, be broken by blowing hot air on to it, though it is not desirable to destroy it completely. A residual froth bed should be left, upon which the concentrate can float. This becomes necessary as the uni-univalent product, which is really a salt, can sometimes dissociate again, if allowed to come into contact with bulk water. If the precautions mentioned in the previous communication are observed, excellent recoveries from very dilute solutions are obtainable. Furthermore, the remarkable degree of selectivity shown by the method in the case of inorganic ions, when starvation amounts of collector are added incrementally, is shown as well in the case of organic ions.

In order to avoid laborious organic analyses, much of the exploratory work was done with dilute solutions of dyes or indicators, usually of a concentration of about 0·01 gm./l. Such solutions could be decolorized completely, sometimes in a matter of minutes, the colour being collected in the froth. Some dyes, having both anionic and cationic centres, could be floated by either cationic or anionic collectors, depending on the *p*H, but others could only be floated by one type of surfactant.

The following experiment will illustrate the selectivity. 3 mgm. each of potassium indigo sulphonate, congo red, methyl orange, bromphenol blue and phenolphthalein were freshly made up into a 400 ml. aqueous solution, and made slightly alkaline with ammonia to make the dyes anionic. This solution was put into a No. 4 sintered glass Buchner funnel. Solid lauryl pyridinium chloride was freshly dissolved in absolute alcohol (2·2 mgm./ml.) and was added in 1 ml. portions, followed by air blown gently through the sintered glass. The dyes were floated in the above order, the distinctive colour of each being clearly perceptible in the froth at each stage. After addition of about 7 ml. of collector, the solution was completely colourless. In a similar experiment, to which Lauth's violet, a cationic dye, had been added, the blue colour of it remained and could not be removed by a cationic collector. If, however, the solution was made slightly acid, *p*H 5, and α-sulpholauric acid added as a collector, the dye was removed by bubbling, leaving the solution colourless.

In working with dyes, it is advisable to use fresh solutions, as dyes have a tendency to form micelles, and old solutions may give misleading results. As it is

only ions which are collected, and as organic substances are usually weakly dissociated, the control of *p*H is very critical. As cationic collectors, the alkyl quaternary ammonium soaps, or the long-chain amines proved most suitable, and as anionic collectors, α-sulpholauric acid or its potassium salt proved most convenient. It is possible to recover the floated organic ion by a double decomposition reaction in a non-aqueous solvent, either benzene or alcohol, each procedure being determined by a knowledge of the chemistry of the species involved.

A number of organic ions, chosen at random, have been floated, establishing the generality of the method. These included picrate, gallate, tetraphenyl boron, and the alkaloids strychnine and brucine. This indicates that the method might be useful in collecting organic ions from natural products, especially as the carbohydrates are not likely to float. It has potential application in concentrating antibiotics and other valuable biological products which are ionizable.

Another possible use is in clarifying solutions. For example, a sugar solution that had been charred to a black molasses residue was dissolved in water to give a dirty brown solution. Using trimethyldodecylammonium chloride as a collector, the colouring matter was floated into the froth, leaving the solution almost colourless.

One of the difficulties in taking the fullest advantage of the selectivity shown by ion flotation in respect of organic ions is that the products are usually colourless, so that it becomes difficult to ascertain when one species is fully collected and the next is about to concentrate. It may become possible to overcome this problem by taking advantage of the large variety of dyestuffs. If these were to be placed in a series of relative floatability, it may be possible to find a dye just intermediate between the two species to be separated. On the introduction of a small amount of such a 'marker dye' into the solution, it would be possible to see when the collection of the first species is finished, by the first appearance of the dye colour in the froth.

F. Sebba

Department of Chemistry,
University of the Witwatersrand,
Johannesburg.

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Dipole Moments of Fluorine Monoxide and Sulphur Tetrafluoride

In setting up a teaching experiment on the dipole moments of gases, we recently took the opportunity to measure the temperature dependence of the polarization of fluorine monoxide (F_2O) and sulphur tetrafluoride (SF_4). The condenser comprised two concentric stainless steel cylinders similar to that described by Groves and Sugden[1]. The capacity of the condenser was about 80 *p*F. and was measured with an accuracy of 1 part in 16,000 by a ratio-transformer bridge. The condenser was mounted in a cylindrical glass vessel which was immersed in the thermostat bath. Capacities were measured with the gases at atmospheric pressure over the temperature-ranges $-78°$ to $+80°$ C. for fluorine monoxide and $-30°$ to $+80°$ C. for sulphur tetrafluoride. The apparatus was calibrated with dry air free of carbon dioxide to eliminate the effect of stray capacity. Fluorine monoxide was made by bubbling fluorine, diluted with nitrogen, through 2 per cent caustic soda solution[2]: sulphur tetrafluoride was obtained through the courtesy of Imperial Chemical Industries, Ltd. (Alkali Division).

The plot of total polarization against reciprocal temperature was not sufficiently accurate to permit extrapolation to infinite temperature. Values of the induced polarization were therefore estimated independently: 5 c.c./mole for fluorine monoxide and 12·5 c.c./mole for sulphur tetrafluoride. Straight lines drawn through these and the experimental points gave the following values of the dipole moments; the errors indicate maximum and minimum slopes of lines drawn through the estimated point at infinite temperature.

Fluorine monoxide $\mu = 0{\cdot}4 \pm 0{\cdot}1$ *D* using $P_\infty = 5$ c.c./mole; sulphur tetrafluoride $\mu = 1{\cdot}0 \pm 0{\cdot}1$ *D* using $P_\infty = 12{\cdot}5$ c.c./mole.

The value for fluorine monoxide is much smaller than that of OH_2 (1·84 *D*) and is thus similar to NF_3 (0·2 *D*) the dipole moment of which is much smaller than that of NH_3 (1·5 *D*). Coulson[3] suggests that the true bond moment of OH in water is 1·08 *D* in the sense $\overset{+}{O}\!\longrightarrow\!H$. The lone pair moment in water must then be 3·19 *D*. If the same lone pair moment is assumed in fluorine monoxide on the grounds that the same angle indicates the same hybridization, then the true O—F bond moment is 2·9 or 2·3 *D* depending on the sense of the overall moment, 0·4 *D*.

Little can be said about sulphur tetrafluoride except that the dipole moment is consistent with the C_{2v} structure which has been proposed[4], and which arises from a trigonal bipyramid (dsp^2 hybridization) when one of the three equatorial positions is occupied by the lone pair. It would, however, be equally consistent with the C_{2v} symmetry which would obtain if the lone pair occupied a polar position.

We are grateful to Dr. M. Fleischmann for his advice and to the Department of Scientific and Industrial Research for a grant to one of us (R. L.).

R. E. Dodd
R. Little

The Chemical Laboratories,
King's College,
Newcastle upon Tyne 1.

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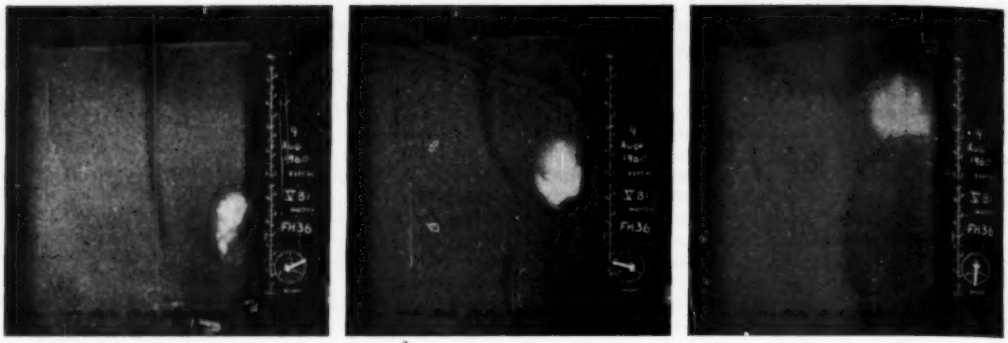
Gas-Flow Patterns in Fluidized Beds

A fluidized bed is sometimes used as a means of carrying out a chemical reaction between a gas and solid particles. In gas-fluidized beds, 'bubbles' form and there is considerable interest in the part played by these in gas/solids in contact. It has been suggested, for example, that some of the gas rises through the bed in the form of bubbles and effectively by-passes the solid particles[1]. Several investigators[1–7] have made various indirect measurements and have concluded that back-mixing of the gas occurs. These processes are important in estimating reaction-rates. So far as we are aware, no one has hitherto observed directly gas-flow patterns within the bed.

We have recently measured directly local gas velocities in a fluidized bed under special conditions.



$t = 0$ $t = 0{\cdot}19$ sec. $t = 0{\cdot}40$ sec.

Fig. 1

The bed was 12 in. wide and 15 in. deep, but only 1 in. from back to front so that it was effectively two-dimensional. The containing walls were made of 'Perspex', and within them 0·46-mm. diameter glass spheres were fluidized by air. Typical 'bubbles' formed and with rear illumination they could be seen in some detail. Near the bottom of the bed a number of small holes were drilled through the wall and into any of these a fine stream of nitrogen peroxide could be injected. With suitable illumination the progress of this coloured tracer stream could be followed through the bed, and by interrupting it periodically, velocities could be measured. The tracer flow-rate was less than 0·5 per cent of the fluidizing gas-rate so that its overall effect was negligible.

The accompanying photographs (Fig. 1) were selected from a film taken at 100 frames/sec. They show events at approximately 0·2-sec. intervals. The air velocity based on the empty cross-section was 0·9 ft./sec. (about 10 per cent above the minimum fluidization velocity) and the 'bubble' velocity is seen to be about 1·3 ft./sec. but it is increasing up the bed. The tracer stream velocity was about 1·6 ft./sec. and gas is seen to be continuously flowing into the void. Other photographs show the tracer flowing into the void as a jet, but it quickly loses its identity and becomes mixed with air. From a 'bubble' filled with this diluted nitrogen peroxide, we have seen the gas diffuse through the surrounding particles, mostly through the top of the void. Thus, gas in the 'bubble' is by no means isolated from the bulk of the bed. Back mixing of the gas by reverse flow does not generally occur, at least during gentle fluidization, but occasionally, when the solids are very violently mixed, they carry tracer gas with them against the general direction of flow; but this is a rare event.

P. N. Rowe
P. F. Wace

Chemical Engineering Division,
Atomic Energy Research Establishment,
Harwell, Didcot.

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Evidence for a Nitric Oxide Complex with Cupric Fluoride

Although it has been recognized for some time that nitric oxide and cupric chloride or bromide combine in non-aqueous solvents to form nitrosyl complexes[1], the composition of these has only recently been determined[2]. The deep blue colour is due to the covalent $CuX_2.NO$ (possibly solvated): this compound dissociates into colourless ions:

$$CuX_2.NO + ROH \rightleftarrows CuX_2 + H^+ + RONO$$
(ROH = alcohol)

and this has been responsible for erroneous structures suggested for the complexes. Unsuccessful attempts have been made to prepare nitrosyl complexes in alcoholic solution from other cupric salts, including the fluoride[3]. Recently, a study of the variation of dissociation constant of the cupric chloride nitrosyls as a function of the dielectric constant of the alcohol has been made[4]: some results are shown in Table 1.

Table 1. Dissociation Constants for $CuCl_2.NO$ in Alcohols

Solvent	K (23° C.)
Methanol	$4{\cdot}2 \times 10^{-3}$
Ethanol	$5{\cdot}4 \times 10^{-3}$
n-Propanol	$5{\cdot}3 \times 10^{-4}$
n-Butanol	$2{\cdot}0 \times 10^{-5}$

The decrease in dissociation is much greater than would be predicted by the Born equation if dielectric constant were the only factor involved. Thus it appeared that if a nitrosyl could be prepared from cupric fluoride it would only be stable in an alcohol such as *n*-butanol. Using anhydrous cupric fluoride, a violet colour is indeed obtained when nitric oxide is bubbled into the butanol solution, and the absorption of the gas is greater than it is in the absence of cupric salt. The colour fades rapidly, and a white precipitate containing copper and fluoride forms. A similar result is obtained with hydrated cupric fluoride. If the solvent used is *t*-butanol, the violet colour is quite stable, and no precipitate forms for at least 10 min. Addition of hydrofluoric acid has no effect on the optical spectrum of the *t*-butanol complex; but when a trace of the acid is added to any other alcohols containing either anhydrous or hydrated cupric fluoride, and nitric oxide bubbled through, the deep blue nitrosyl colour forms immediately and is quite stable. Fig. 1 shows the close resemblance of the absorption spectra of some of the fluoride nitrosyls to the cupric bromide nitrosyl formed in *n*-butanol.

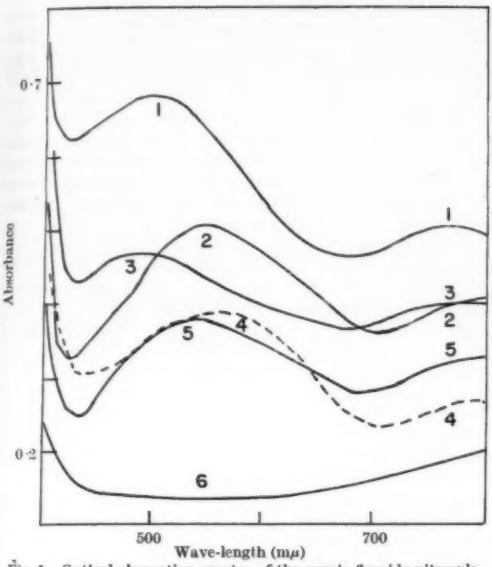

Fig. 1. Optical absorption spectra of the cupric fluoride nitrosyls in alcohols
1, Ethanol; 2, *n*-butanol; 3, *t*-butanol; 4 (dotted line), $CuBr_2.NO$ in *n*-butanol, shown for comparison; 5, *n*-propanol; 6, CuF_2 hydrate in *n*-propanol

There is a gradual shift in the position of the absorption maximum of the complex as the solvent is changed. Table 2 shows wave-lengths of maximum absorption for the three nitrosyl complexes in alcohols[4].

Table 2. Position of Absorption Maxima

Solvent	$CuF_2.NO$	Max. λ(mμ) $CuCl_2.NO$	$CuBr_2.NO$
Ethanol	499	550	570
n-Propanol	551	550	570
n-Butanol	551	550	565
t-Butanol	475	—	530

Methanol is the only alcohol examined so far in which the nitric oxide–cupric fluoride cannot be formed in the presence of hydrofluoric acid: only a transient yellow coloration is obtained. The nitrosyl solutions are not stabilized by lithium or sodium fluorides as they are by hydrofluoric acid. The role of the acid is merely to depress the ionization of the covalent nitrosyl.

R. T. M. Fraser

Department of Chemistry,
University of Ottawa.

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Separation of Amino-acid Mixtures on Sulphonated Polystyrene Resin-loaded Papers

The high resolving powers of columns of cross-linked sulphonated polystyrene resins when applied to the separation of mixtures of amino-acids has been demonstrated by the classic work of Moore and Stein[1] and afterwards by other workers[2,3].

For rapid qualitative separations a paper chromatographic technique offers definite advantages. In addition to its simplicity of operation is the ability to: (*a*) detect the separated zones on the paper, thus rendering elution unnecessary; (*b*) develop several chromatograms side by side; (*c*) record simply the rate of movement of a zone relative to that of the solvent front; and (*d*) combine two separate developments on the same sheet of paper as a two-dimensional chromatogram.

The advent of the ion-exchange papers has made it possible to realize the advantages of both ion-exchange method and paper chromatographic technique. The object of the work presented here is to compare in a purely qualitative manner the resolving powers of ion-exchange resin in column and paper form.

Separations of the amino-acids on ion-exchange cellulose papers have previously been described[4], as also have some limited separations on ion-exchange resin-loaded papers, but so far the resolution on the latter materials has not approached that possible on resin columns[5,6].

The most satisfactory results were obtained on a paper (patent applied for), made specially in these Laboratories, containing 'Zeokarb 225 (*WR* 1·5–2·0)' resin in a form particularly suited to the requirements of chromatographic separations.

A number of other sulphonated polystyrene resin-loaded papers were also investigated, including some commercially available papers, but were found to be of limited use for critical separations.

1 μgm. of each of sixteen amino-acids was applied as separate spots to a sheet of the resin-loaded paper which had previously been equilibrated with 0·2 *M* *p*H 3·1 buffer[1]. The equilibrated paper was carefully blotted to remove excess liquid and the amino-acids were applied to the wet paper. The chromatogram was developed in the 0·2 *M* *p*H 3·1 buffer by a descending technique for a period of 90 min., after which the dried paper was sprayed with a 0·2 per cent solution of ninhydrin in acetone containing 0·1 per cent collidine[7]. The pattern of amino-acids which resulted is illustrated in Fig. 1.

A separation of amino-acids on a column of 'Zeokarb 225 (*WR* 1·5–2·0)' through 200-mesh resin, developed according to the Moore and Stein technique, is illustrated in Fig. 2.

10 mgm. of amino-acid mixture was applied to the column, and the complete run took 50 hr.

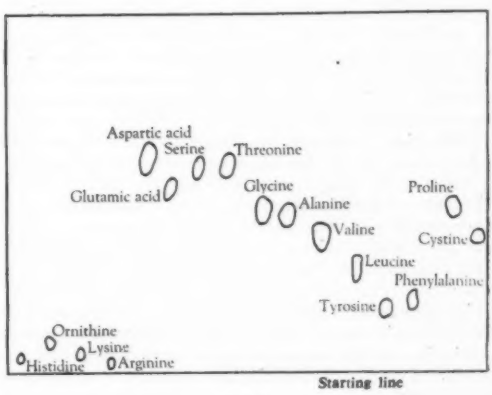

Fig. 1

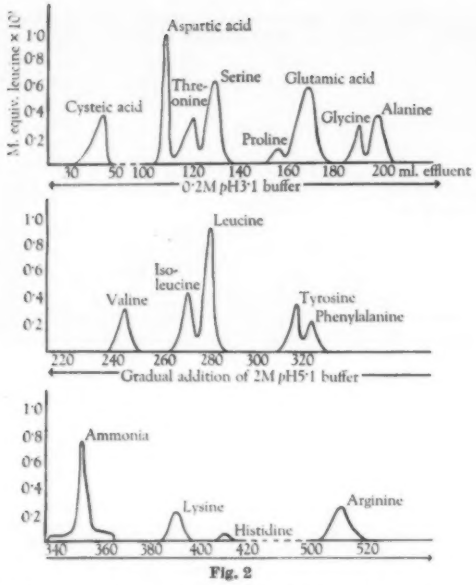

Fig. 2

Although the gradient elution of the column technique has been replaced on the paper by a simple elution with a single buffer, the sequences on the two forms of the resin, with the possible exception of the basic group of amino-acids, are comparable, and such a comparison is made in Table 1.

Table 1. A Comparison of the Sequence of Amino-acids on Column and Papers containing 'Zeokarb 225' Resin

Sequence of decreasing rate of movement	
Sulphonated polystyrene resin column (4·5 per cent cross-linked). Developed according to Moore and Stein (ref. 1)	Sulphonated polystyrene resin-loaded paper. Developed in 0·2 *M* *p*H 3·1 buffer
Asp	Asp
Thr	Thr
Ser	Ser
Pro	Glu
Glu	Pro
Gly	Gly
Ala	Ala
Val	Val
Leu	Cys
Tyr	Leu
Phe	Phe
Orn	Tyr
Lys	Orn
His	Lys
Arg	His
	Arg

It may be seen that some differences in sequence on the two forms of the resin occur and that these are mainly associated with a reversal of the tyrosine–phenylalanine and the proline–glutamic acid groups. These differences can probably be attributed to the influence of the cellulose matrix on separations on the resin-loaded papers.

With the exception of the above differences the high resolution of the column technique is matched by that of the paper, and because the latter can be achieved without elution the time required for such a separation is considerably reduced.

A property of the ion-exchange paper which has not been used in the above separation is that associated with the cellulose matrix itself when the ion-exchange characteristics are suppressed. Separations describing the use of a new two-dimensional technique in which ion-exchange and 'partition' techniques are combined on the ion-exchange cellulose papers have been described[4,5].

In the case of the sulphonated polystyrene resin-loaded paper the separation of the amino-acids achieved under ion-exchange conditions ideally combines with that achieved under 'partition' conditions. This is because the sequences of amino-acids produced by the two sets of conditions are very different. This is a point of difference from the ion-exchange cellulose papers and is due to the different matrixes associated with the two types of exchanger. A separation illustrating the new two-dimensional method is shown in Fig. 3.

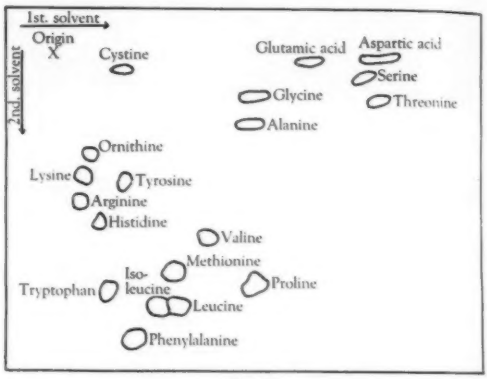

Fig. 3

In this the first development was carried out with 0·2 *M* *p*H 3·1 buffer on the dry sodium form of the paper and the second development in *m*-cresol – 0·3 per cent ammonia. The ammonia solution, present in the bottom of the chromatography tank, assured a suppression of the effect of ion-exchange characteristics by producing an anionic form of the amino-acids. The paper was equilibrated over the ammonia for 1 hr. before introduction of the second solvent. The complete separation was achieved in a single day.

The most striking feature of the separation illustrated in Fig. 3 is the absence of amino-acid spots along the diagonal of the paper through the origin. A diagonal distribution of spots and consequently a lack of high resolution are common features of many conventional two-dimensional chromatograms. By completely breaking up such a distribution and by adding to the high resolving power of the resin itself the new two-dimensional technique on sulphonated polystyrene resin-loaded paper probably provides the highest degree of resolution of amino-acids yet obtained in a single chromatographic analysis.

A fuller account of the analysis of amino-acid mixtures on different types of ion-exchange papers is being prepared and will be published elsewhere.

I wish to thank the directors of W. and R. Balston. Ltd., for permission to publish this communication.

C. S. Knight

Whatman Research Laboratories,
Springfield Mill, Maidstone, Kent.

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BIOCHEMISTRY

Hexosamine Synthesis by Cell-free Extracts of Various Bacteria

An enzyme system catalysing the synthesis of glucosamine-6-phosphate from glutamine and fructose-6-phosphate or glucose-6-phosphate has been demonstrated in *Neurospora crassa*[1,2], rat liver[2,3] and *Escherichia coli*[3]. Ghosh *et al.*[3], who purified the enzyme from these various sources and studied its properties, have called it L-glutamine-D-fructose-6-phosphate transamidase. We have determined transamidase activity in fresh, cell-free extracts of *Bacillus cereus*, *Micrococcus lysodeikticus*, *Aerobacter aerogenes* and *Escherichia coli*. Results indicate that, for a given weight of cells, activity is higher in Gram-positive than in Gram-negative bacteria, and for a given organism, activity increases markedly during the lag and early exponential phases of growth, but decreases during the stationary phase. The high transamidase activity associated with dividing cells is probably related to the synthesis of cell-wall basal structure[4], which contains amino-sugars. During these investigations, using a system with standard conditions of *p*H, substrate concentrations and reaction time at 37°, it was found that the amount of glucosamine-6-phosphate synthesized rarely exceeded 25 per cent theoretical, and frequently was not proportional to enzyme concentration when different volumes of the same cell extract were tested in parallel. An attempt was made to explain these findings.

Cell-free extracts were prepared from *Bacillus cereus* vegetative cells suspended in water (20–50 mgm. dry weight/ml.) by disintegration with ballotini beads in a Mickle disintegrator[5] and centrifugation of the homogenates. The activity of an extract prepared in this way was equivalent to that obtained from the same weight of cells disintegrated in a Hughes press[6]. The rates of formation of hexosamine in the presence of extract and various amounts of glucose-6-phosphate (extract contained phosphoglucoisomerase[2]) and glutamine at *p*H 6·5 are shown in Fig. 1. Control tubes containing heat-inactivated extract and substrates were included, and hexosamine was determined in tests and controls by the method of Rondle and Morgan[7] modified by the use of carbonate–bicarbonate buffer[8]. In all cases hexosamine increased to a maximum not exceeding 25 per cent theoretical (based on amount of glucose-6-phosphate present) and then decreased. Similar results were obtained when reaction mixtures were buffered at *p*H 7·5. Apparently the low maximum yield of product was not due to enzyme inactivation during incubation since hexosamine was synthesized when glutamine was added to glucose-6-phosphate + extract + buffer preincubated for 1 hr. at 37°. Nor was destruction of substrates by glutaminase and phosphatase activities in the extract sufficient to explain the results. Loss of hexosamine-reacting material after the maximum had been reached was not due to formation of *N*-acetylhexosamine derivatives or incorporation into a larger molecule since no increase in hexosamine was found after hydrolysis with 6 *N* hydrochloric acid. Comb and Roseman[9] purified and studied the properties of an enzyme in *Escherichia coli* which deaminated glucosamine-6-phosphate releasing fructose-6-phosphate and ammonia; the reaction was reversible. An enzyme with similar properties was present in extracts of the bacteria mentioned

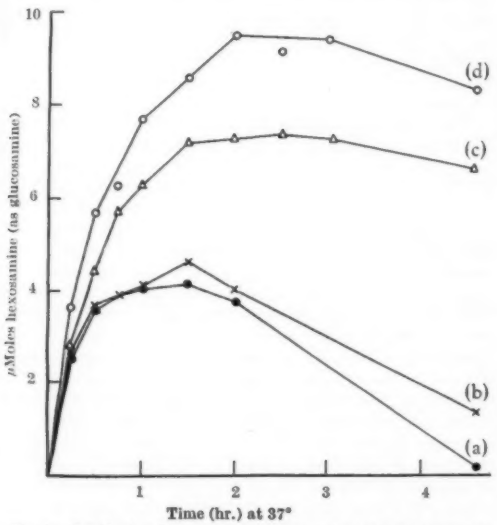

Fig. 1. Synthesis of hexosamine by an extract of disintegrated *Bacillus cereus* cells. Reaction mixtures (1 ml.) contained 0·3 *M* phosphate buffer, *p*H 6·5 (0·2 ml.), extract (from 18 mgm. dry weight cells) and substrates as follows (μmoles):

	(*a*)	(*b*)	(*c*)	(*d*)
Glucose-6-phosphate	20	20	40	40
Glutamine	20	50	40	100

Samples (0·1 ml.) removed immediately and at intervals during incubation at 37° were acidified with acetic acid, heated at 100° for 2 min. and diluted to 3 ml. Hexosamine was determined in the supernatant fluids (1 ml.)

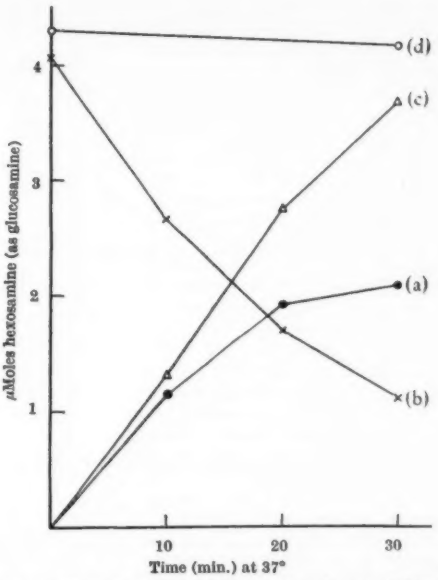

Fig. 2. Comparison of transamidase and deaminase activities in an extract of *Bacillus cereus* cells. Reaction mixtures (0·5 ml.) contained 0·4 *M* phosphate buffer (0·2 ml.), extract (from 6 mgm. dry weight cells) and other additions as follows (μmoles):

	(*a*)	(*b*)	(*c*)	(*d*)
Glucose-6-phosphate	15	—	15	15
Glutamine	25	—	25	—
Glucosamine-6-phosphate	—	4·3	—	4·3
Ammonium chloride	—	—	375	375
Versene	—	—	15	15

Samples (0·1 ml.) removed and treated as in Fig. 1

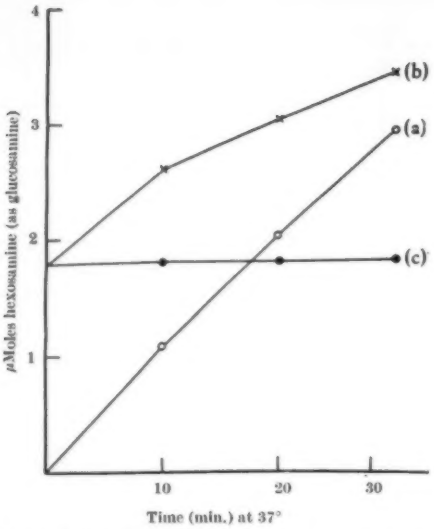

Fig. 3. Effect of added glucosamine-6-phosphate on hexosamine synthesis in the presence of excess ammonium salt. Reaction mixtures (0·5 ml.) contained 0·4 *M* phosphate buffer, *p*H 7·5 (0·12 ml.), extract (from 14 mgm. dry weight *Bacillus cereus* cells), versene (15 μmoles), ammonium chloride (250 μmoles), glucose-6-phosphate (10 μmoles) and other additions as follows (μmoles):

	(a)	(b)	(c)
Glucosamine-6-phosphate	—	1·8	1·8
Glutamine	20	20	—

Samples (0·1 ml.) removed and treated as in Fig. 1

above and its activity was maximal near *p*H 8·5 but appreciable at *p*H 6·5–7·5. Using the same amount of *B. cereus* cell extract at *p*H 7·5, the rate of disappearance of hexosamine from a reaction mixture containing glucosamine-6-phosphate was compared with the rate of hexosamine synthesis in the presence of glucose-6-phosphate and glutamine (Fig. 2). Results indicated that the apparently restricted synthesis of hexosamine was due to destruction of the product by deaminase activity. Attempts were made to inhibit the degradation reaction. Deaminase activity was markedly reduced by a mixture of disodium ethylenediamine tetraacetic acid (versene) ammonium salt in excess and glucose-6-phosphate, whereas transamidase activity increased in the presence of ammonium salt + versene (Fig. 2) and was proportional to the volume of extract added when reaction mixtures were incubated for 15–30 min. at 37°. A small synthesis of hexosamine occurred when extract was incubated with glucose-6-phosphate and excess ammonium salt at *p*H 7·5, but this did not account for the increased transamidase activity. However, it was shown that deaminase activity was not completely inhibited under these conditions, by measuring transamidase activity in the presence and absence of added glucosamine-6-phosphate; inhibition was less when the concentration of hexosamine in reaction mixtures increased (Fig. 3).

Under the conditions described, the direct, accurate measurement of L-glutamine-D-fructose-6-phosphate transamidase activity in crude bacterial extracts is not possible owing to the presence of glucosamine-6-phosphate deaminase. Methods for the purification of the two enzymes have been described by other workers[3,9], and it is possible that quantitative separation of the transamidase could be achieved. However, the instability of the enzyme[1–3] makes this a difficult problem.

R. E. Strange
F. A. Dark

Microbiological Research Establishment,
Porton, Nr. Salisbury,
Wilts.

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Heterogeneity of Liver Lecithin isolated by Chromatography on Silicic Acid Columns

The recent report by Collins[1] of the heterogeneity of lecithin labelled with phosphorus-32 is supported by results obtained by us as part of an investigation of the effect of ethionine on the incorporation of phosphorus-32 into the liver phosphatides of rats. This will be reported in full elsewhere but an experiment on normal rats, which is relevant to the work of Collins, is described here.

Three rats were each injected intraperitoneally with 4 μc. of labelled orthophosphate. Three hr. later, the rats were killed and their livers were removed. The lipids from the combined livers were extracted and freed from water-soluble components containing radioactive phosphorus by a procedure similar to that described by Fishler and Chaikoff[2], except that the ether extract of the liver lipids was washed with 0·1 *N* hydrochloric acid and 0·01 *N* sodium chloride[3], rather than with a solution of unlabelled sodium phosphate.

The total phosphatides were first separated from the other lipids in the extract on a silicic acid column[4] and were then fractionated on a second column of silicic acid using mixtures of chloroform and methanol as the eluting solutions[5,6]. The silicic acid columns were 'aged' before use as described by Gray and Macfarlane[7] to minimize breakdown of acetal phosphatides. The fractions from the column were analysed for choline, amino-nitrogen and inositol, as well as for total phosphorus and esterified fatty acids. The fractionation, on the basis of the phosphorus analyses, is shown by the elution curve in Fig. 1. The main component eluted by the 4 : 1 chloroform/methanol mixture is a mixture of serine and ethanolamine phosphatides, and the main component eluted by the 1 : 1 chloroform/methanol mixture is lecithin. Inositol phosphatide is eluted immediately before the lecithin and, in this particular fractionation, is incompletely separated from it. This pattern of elution of rat liver phosphatides is the same as that described by Hanahan *et al.*[6].

The specific activities of the successive fractions collected from the column during the elution of the lecithin component are also shown in Fig. 1. There is a continuous rise in the specific activity as the lecithin is eluted. This increase in specific activity could be caused by contamination of the lecithin with the components which either preceded or followed

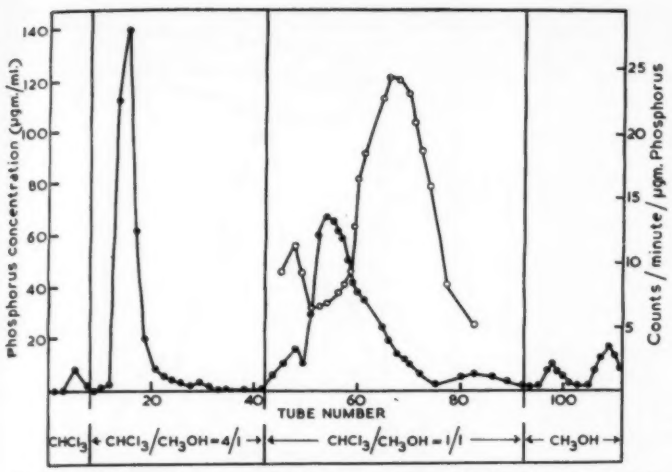

Fig. 1. Separation of liver phosphatides on a column of silicic acid (Mallinckrodt's analytical reagent, 100 mesh). Load, 25 mgm. per gm. silicic acid. Flow-rate, 0·5–1 ml. per min. The eluting solvents were mixtures of chloroform and methanol in the ratios shown in the figure. 10 ml. or 20 ml. samples of eluate were collected. —●—, phosphorus concentration in μgm./ml.; —○—, counts/min./μgm. phosphorus

it from the column. However, the specific activity of the inositol phosphatide fraction, measured in tube 48, was 12·0 and that of the uncharacterized component eluted immediately after the lecithin, measured in tubes 80–85, was 6·3. Such an explanation is therefore unlikely. A similar rise in specific activity, not shown here, was observed as the mixed serine and ethanolamine phosphatides were eluted.

The combined fractions in the tubes 50–55 (fraction *A*) and in tubes 60–72 (fraction *B*) from the lecithin peak were examined further. Acetal phosphatides, measured by the method of Gray and Macfarlane[7], formed 3 per cent of the total phosphatides in fraction *A* and were absent from fraction *B*. The phosphorus/choline/esterified fatty acid ratio in fraction *A* was 1/0·97/1·90 and that in faction *B* was 1/0·92/1·94. After methanolysis of the fractions, the methyl esters of the fatty acids were analysed by gas-liquid chromatography (Table 1).

Table 1. Percentage Fatty Acid Composition of Lecithin Sub-Fractions *A* and *B*

Fatty acid (trivial names)	No. of carbon atoms and double bonds	Retention time relative to stearate	Fraction *A*	Fraction *B*
Myristic	14–0	0·204–0·215	—	0·46
—	15–0	0·310–0·314	—	0·42
Palmitic	16–0	0·456–0·461	23	33·2
Palmitoleic	16–1	0·515–0·520	0·72	1·98
—	17–0 (?)	0·665–0·690	0·72	0·68
—	17–? un.	0·830–0·833	0·50	—
Stearic	18–0	1·000	44·4	16·3
Oleic	18–1	1·10–1·11	4·53	15·4
Linoleic	18–2	1·34–1·35	5·81	21·2
Arachidonic (?)	20–4 (?)	3·52–3·56	20·2	10·3

The fatty acids in fractions *A* (tubes 50–55) and *B* (tubes 60–72) were converted to the methyl esters by refluxing the fractions with 1·3 *N* hydrochloric acid in a dry methanol/benzene mixture for 2 hr. The methyl esters were separated on the Pye argon gas chromatograph at 175° C. and with an argon flow-rate of 50–60 ml./min. The stationary phase was the adipate ester of polyethylene glycol (10 per cent on acid-washed, 100–120 mesh, 'Celite'). The relative retention time for the methyl ester of arachidonic acid on this column is normally 3·22. The identification of the C20–4 component as arachidonic acid is probably correct, however, since its relative retention time on a second column with 'Apiezon *L*' as stationary phase was 1·48, the usual value for the methyl ester of arachidonic acid. Under the above experimental conditions the determination of unsaturated acids with C22 and upwards is unsatisfactory and values for these acids are not included in Table 1. They were present in both fractions as minor components, the amounts in fraction *A* being greater than those in fraction *B*.

The results show marked differences in the fatty acid composition of the two fractions. For example, stearic and arachidonic acids are the main saturated and unsaturated acids in fraction *A*, whereas in fraction *B* the main saturated acid is palmitic acid and the main unsaturated acid is linoleic acid. The combined percentage of mono- and poly-saturated fatty acids is greater in fraction *B* than in fraction *A*. This pattern has been confirmed in further work.

As prepared by silicic acid chromatography, rat liver 'lecithin' therefore appears to be heterogeneous. Its heterogeneity can be expressed both in terms of differences in fatty-acid composition and in the rate of turnover of the 'lecithin' phosphorus.

P. M. Harris
D. S. Robinson

Medical Research Council External Staff,
Sir William Dunn School of Pathology,
University of Oxford.

G. Getz*

Department of Biochemistry,
University of Oxford.

* Present address: Department of Chemical Pathology, University of the Witwatersrand, Johannesburg.

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Transforming Activity of Deoxyribonucleic Acid labelled with 5-Bromouracil

Ephrati-Elizur and Zamenhof[1] demonstrated that deoxyribonucleic acid from *Bacillus subtilis* grown in 5-bromouracil-supplemented media contained this analogue and retained its transforming activity. It thus became important to determine the specific transforming activity for purified fractions of each of the three molecular variants of deoxyribonucleic acid which can be extracted from organisms treated with 5-bromouracil: (1) unlabelled deoxyribonucleic acid

molecules, and those in which 5-bromouracil has been substituted for thymine in (2) one strand ('unifilar' labelling) or in (3) both strands ('bifilar' labelling) of the double helix. Two strains of *Bacillus subtilis*, wild type[2] and uracil-requiring strain 265 (kindly supplied by Dr. R. Guthrie), served as the donors of deoxyribonucleic acid. These were first grown with aeration at 37° C. for 4 hr. in nutrient broth supplemented with thymidine (10 μgm. per ml.), and afterwards incubated for 2–3 generations in minimal broth[3] containing 2–5 μgm. 5-fluorodeoxyuridine (serving as the thymidylic acid synthetase inhibitor[3]), 25 μgm. uridine (to suppress the conversion of 5-fluorodeoxyuridine to 5-fluorouracil and 5-fluorouridine) and 50–250 μgm. 5-bromodeoxyuridine per ml. Under these conditions, similar to those first employed for *Escherichia coli* by Lorkiewicz and Szybalski[4], the molar ratio of 5-bromouracil to 5-bromouracil + thymine (5-bromouracil substitution) in non-fractionated deoxyribonucleic acid varied from 35 to 61 per cent as assessed by chromatographic determination[5].

Employing another procedure, with aminopterin as inhibitor of thymidylic acid synthesis, the wild-type strain was reported to incorporate essentially no 5-bromouracil, while analogue substitution in strain 265 was 12 per cent[1]. Highly polymerized deoxyribonucleic acid was isolated from cells lysed by lysozyme (100 μgm./ml.) and sodium laureate (5 per cent), deproteinized by the chloroform–butanol or phenol[5] procedures and freed of ribonucleic acid by

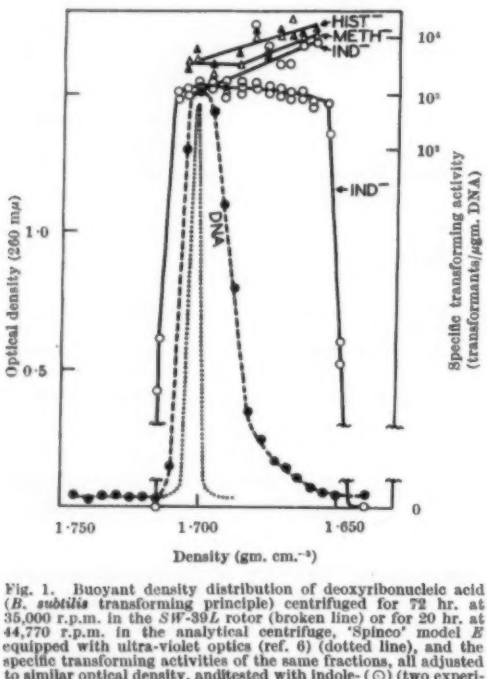

Fig. 1. Buoyant density distribution of deoxyribonucleic acid (*B. subtilis* transforming principle) centrifuged for 72 hr. at 35,000 r.p.m. in the *SW*-39*L* rotor (broken line) or for 20 hr. at 44,770 r.p.m. in the analytical centrifuge, 'Spinco' model *E* equipped with ultra-violet optics (ref. 6) (dotted line), and the specific transforming activities of the same fractions, all adjusted to similar optical density, and tested with indole- (○) (two experiments), methionine- (△) and histidine-16- (▲) requiring recipient strains of *B. subtilis* (0·1 μgm. deoxyribonucleic acid, 10^8 cells per ml. medium). Each point corresponds to a 3- or 9-drop fraction (30–90 μl.), out of the total 3 ml. used for centrifugation. We are indebted to Mr. Carl Schildkraut of the Department of Chemistry, Harvard University, for the analytical centrifuge runs and to Drs. J. Marmur and J. Eigner and Prof. P. Doty for the help and hospitality extended to one of us (W. S.) during the performance of these determinations

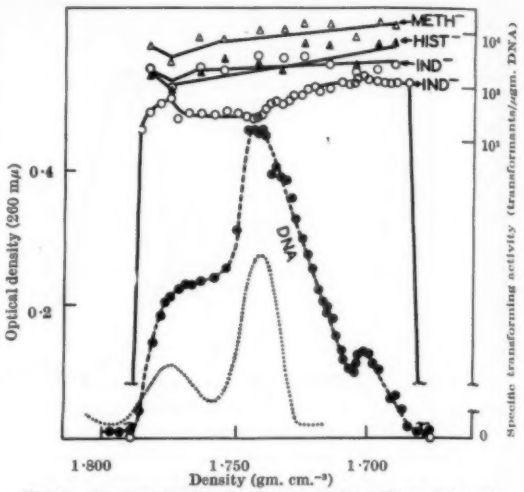

Fig. 2. Buoyant density distribution of deoxyribonucleic acid labelled with 5-bromodeoxyuridine, extracted from *B. subtilis* grown for approximately two generations on 5-bromodeoxyuridine, 5-fluorodeoxyuridine, uracil-supplemented minimal medium. For explanation of curves, see Fig. 1. The left peak (1·776 gm. × cm.⁻³) corresponds to 70·5 per cent 'bifilar' 5-bromodeoxyuridine labelling while the centre peak (1·742 gm. × cm.⁻³) represents the 'hybrid' molecule (70·5 per cent 'unifilar' labelling). Since guanine + cytosine comprise 42 moles per cent of *B. subtilis* deoxyribonucleic acid bases (ref. 11), overall incorporation of bromouracil into the deoxyribonucleic acid represented by these density peaks was calculated to amount to 46·7 per cent, which figure agrees with the results of chromatographic 5-bromouracil determinations (ref. 5) on non-fractionated deoxyribonucleic acid. The small peak on the right (1·702) probably corresponds to deoxyribonucleic acid free of 5-bromodeoxyuridine extracted from the small proportion of cells which were non-viable or died at the time of transfer to 5-bromodeoxyuridine-supplemented medium

ribonuclease treatment (37°, 2 hr.) and overnight dialysis. The density gradient centrifugation procedure[6] was employed for the fractionation of deoxyribonucleic acid labelled with 5-bromodeoxyuridine. When centrifuged for 72 hr. at 35,000 r.p.m. in a 'Spinco' swinging-bucket rotor, the normal, deoxyribonucleic acid molecules free from 5-bromodeoxyuridine converged to a position in the cæsium chloride gradient corresponding to a density of approximately 1·703 gm. cm.⁻³ (Fig. 1). This was determined either by analytical cæsium chloride centrifugation[6] or by measuring the density and deoxyribonucleic acid content (optimal density at 260 mμ) of all the fractions, 30–90 μl. each, collected by the method described by Szybalski[7]. Specific transforming activity for 3 genetic markers was determined for each fraction, employing indole, methionine, and histidine-deficient receptor strains of *B. subtilis*. The methods for preparation and treatment of recipient cells were essentially the same as described by Spizizen[8]. As can be inferred from Fig. 1, the specific transforming activity, expressed as the number of transformant colonies per μgm. deoxyribonucleic acid (using a standard number of 10^8 recipient cells per ml., under standard conditions), was practically identical for all the fractions of normal, deoxyribonucleic acid free from 5-bromodeoxyuridine.

Similar determinations were performed on 5-bromodeoxyuridine-labelled, cæsium-chloride centrifuged and fractionated transforming deoxyribonucleic acid. The fact that this labelled deoxyribonucleic acid formed bands at only three density-levels, corresponding to the parental, and hypothetical

'unifilarly' ('hybrid'), and 'bifilarly' labelled molecules, is consistent with the hypothesis of semi-conservative replication of *B. subtilis* deoxyribonucleic acid, by analogy with the data of Meselson and Stahl[6]. As briefly reported earlier (indole marker)[8], and as can be seen in Fig. 2, heavy labelling with 5-bromodeoxyuridine of the transforming deoxyribonucleic acid in one (1·742 gm. cm.$^{-3}$ peak) or both (1·776 gm. cm.$^{-3}$ peak) strands did not significantly affect the specific transforming activity for the three markers tested. The labelled deoxyribonucleic acid used in this experiment was highly polymerized with sedimentation constant $S_{20w} = 44s$ (corrected for deoxyribonucleic acid concentration equal to 0), and essentially free of any denatured material, as inferred from the shape of the 'melting' profile[9,10].

In summary, it might be concluded that transforming *B. subtilis* deoxyribonucleic acid molecules retain their biological activity even after extensive 'unifilar' or 'bifilar' labelling with 5-bromodeoxyuridine, as assessed with three genetic markers.

This work was supported by research grants from the National Science Foundation, *G*-7021 to W. Szybalski, and *G*-4337 to S. Zamenhof.

Wacław Szybalski
Z. Opara-Kubinska
Z. Lorkiewicz

McArdle Memorial Laboratory,
University of Wisconsin,
Madison 6,
Wisconsin.

Erela Ephrati-Elizur
Stephen Zamenhof

Department of Biochemistry,
College of Physicians and Surgeons,
Columbia University,
New York 32,
New York.

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Crystallization of the Complex of D-Amino-Acid Oxidase and Benzoate

We have been investigating D-amino-acid oxidase apo-protein, flavin adenine dinucleotide and the substrate with reference to complex formation[1,2]. In the previous communication[1], it was reported that the addition of the apo-protein to flavin adenine dinucleotide causes a shift and decrease of the absorption peak at 450 mμ of the latter. It was also found that the co-existence of benzoate, a substrate-substitute, and the apo-protein causes further marked

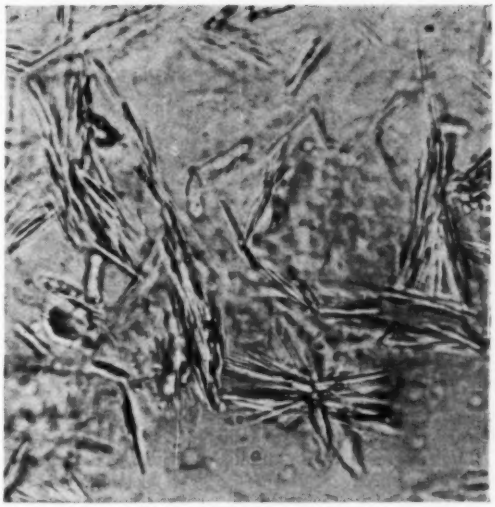

Fig. 1. Crystal of the complex of D-amino-acid oxidase holoenzyme and benzoate. (× 1,000)

changes in the absorption spectrum of the flavin adenine dinucleotide. Two important changes were especially noted: (1) the intensity of the absorption at 450 mμ is further decreased and that at 485 mμ is increased to form a shoulder, and (2) a new absorption peak appears at 465 mμ. These results indicate that both the apo-protein and the benzoate combine with two different binding sites of the *iso*alloxazine nucleus of flavin adenine dinucleotide. On the other hand, the fact that benzoate competes with the substrate, D-amino-acid[3,4], indicates combination between benzoate and the apo-protein. Hence it may be considered that the apo-protein, flavin adenine dinucleotide and benzoate combine to form a tight complex[5].

We have now isolated the complex of D-amino-acid oxidase apo-protein, flavin adenine dinucleotide and benzoate. Excess of both flavin adenine dinucleotide and benzoate was mixed with the apo-protein dissolved in pyrophosphate buffer (*M*/60, *p*H 8·3) at 0°. Then, ammonium sulphate to 0·2 saturation was added to this mixture, bringing the *p*H to 5·1. A yellow precipitate was obtained by centrifugation. This precipitate was dissolved in pyrophosphate buffer (*M*/60, *p*H 8·3) and precipitated by ammonium sulphate at *p*H 5·1. The precipitate was dissolved in its own volume of pyrophosphate buffer (*M*/60, *p*H 8·3), and allowed to stand at room temperature. Needle-shaped crystals then appeared (Fig. 1).

The absorption spectrum of the crystallized preparation is shown in Fig. 2, curve I. The shape of this absorption spectrum is identical with that of flavin adenine dinucleotide co-existing with both the apo-protein and benzoate[1].

The presence of benzoate in this crystal was assumed, and the benzoate was determined. The solution of the crystal was acidified with dilute hydrochloric acid, and benzoic acid was extracted with ether, from which it was re-extracted with 0·01 *N* aqueous potassium hydroxide. For the estimation of benzoate, its absorption at 225 mμ was measured. The presence of benzoic acid in the solution was shown by Folin's reagent after oxidation by hydrogen peroxide. The results showed that the

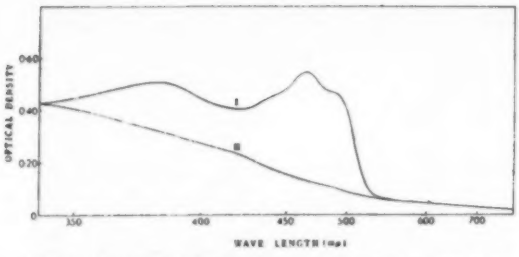

Fig. 2. Spectrum of the complex of D-amino-acid oxidase holoenzyme and benzoate. Curve I, spectrum of the solution of the crystal; the crystal was dissolved in 0·05 *M* potassium phosphate buffer, *p*H 6·3; curve II, spectrum of the same solution 5 min. after the addition of an excess of D-alanine. The spectra were obtained with a Hitachi recording spectrophotometer at room temperature

crystal contained equimoles of the apo-protein, flavin adenine dinucleotide and benzoate.

The enzyme activity of this crystallized sample was also examined. Complete decolorization was demonstrated by the addition of excess D-alanine, as shown in Fig. 2, curve II. With excess D-alanine, this sample showed the same turn-over as that of the apo-protein reported previously[6].

It was also found that when an excess of D-alanine was added to the sample, and the enzyme was precipitated by ammonium sulphate, benzoate was found in the supernatant.

From these results, it may be concluded that the crystal we obtained is the complex of reconstructed D-amino-acid oxidase holoenzyme and benzoate, and that benzoate acts as a hinge to form a tight complex of the apo-protein and flavin adenine dinucleotide without any denaturation of the active core of the apo-protein, and benzoate, a substrate-substitute, can easily be exchanged by the real substrate, D-amino-acid. Hence it is considered that the apo-protein, coenzyme and the substrate combine with each other like three corners of a triangle.

In this connexion, we have compared our crystal with 'holoenzyme of D-amino-acid oxidase' obtained by Kubo *et al.*[7] and Massey and Palmer[8]. Kubo *et al.*[7] reported that they obtained the holoenzyme crystal without splitting off its prosthetic group. We have examined the absorption spectrum of the solution of the crystal obtained by Kubo's method and we find a shoulder at 485 mμ. This result, together with the fact that benzoate was used during the preparation procedure of their enzyme, suggested that the benzoate may still be present in the enzyme sample. Analysis showed indeed that this sample also contains equimoles of the apo-protein, flavin adenine dinucleotide and benzoate.

Recently, Massey and Palmer[8] reported that they obtained a crystal by further purification of Kubo's sample using a calcium phosphate gel-cellulose column and that they removed iron-containing impurities from the sample. This purification was repeated in our laboratory, and it was also found that the treatment by calcium phosphate gel-cellulose chromatography could be simplified by the following treatment: the mixture of calcium phosphate gel and cellulose powder (1 : 30, w/w) was added to the enzyme sample prepared by the method of Kubo *et al.*[7], and stirred at *p*H 7·1. Then the *p*H was brought to 5·1 and the mixture centrifuged at 0°. The reddish-brown precipitate was removed. Repeating this procedure twice, a clear yellow supernatant was obtained. A crop of needle-shaped crystals was obtained from the supernatant.

The absorption spectrum of the crystallized sample obtained by the method of Massey and Palmer[8] and that obtained by the simplified method were identical with that of our crystal synthesized from the apo-protein, flavin adenine dinucleotide and benzoate (Fig. 1, curve I).

The quantity of benzoate in this crystallized sample was also estimated, and it was found that equimoles of the apo-protein, coenzyme and benzoate were present in the sample.

From these results, it is considered that the crystal of 'D-amino-acid oxidase' obtained by Kubo *et al.* and also that by Massey and Palmer are not the enzyme itself, but a complex of D-amino-acid oxidase holoenzyme and benzoate.

Kunio Yagi
Takayuki Ozawa
Minoru Harada

Department of Biochemistry,
School of Medicine,
Nagoya University,
Nagoya, Japan.
July 24.

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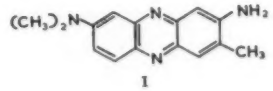
The Phenazinimines: a Possible Explanation of Anomalies associated with Neutral Red

D. L. Vivian and Morris Belkin in 1956 reported[1] that they had synthesized a compound which from the 'unambiguous' method used[2] should be 2-amino-8-dimethylamino-3-methylphenazine:



I

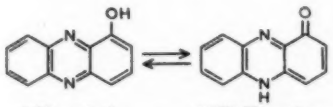
This is the accepted structure for the colour base of neutral red, and a compound having this configuration, when converted to its hydrochloride, should show the normal neutral red behaviour of acting as a vital stain, that is, should stain living cells without killing them. As stated by Vivian and Belkin, however, the compound actually obtained, although giving an analysis agreeing with I, when converted to its hydrochloride, did not show the characteristic vital staining behaviour of neutral red. It also differed from the colour base obtained by removing the hydrogen chloride from a sample of commercial neutral red through chromatographic adsorption on basic alumina, in that it had a very different melting point, as well as in giving a brown colour with concentrated sulphuric acid, instead of the deep green colour which the commercial base forms. Still another point of difference is that the free base from the commercial neutral red fluoresces very slightly if at all in ether

solution, while the more soluble synthetic fluoresces a very strong green under ultra-violet illumination.

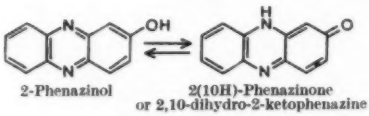
It was found possible by solution in sulphuric acid to convert the new base into one which did behave as the base obtained from commercial neutral red, and also gave the characteristic vital staining of cells from two varieties of *Amoeba* and from sarcoma 37 ascites tumour when it was converted to its hydrochloride, but no attempt was made in this first report to arrive at an explanation of the true nature of the new compound.

Now, however, as a result of further consideration of the problem, together with that of some experimental facts already known, but with which the authors were unacquainted at the time, I have formulated the following possible explanation:

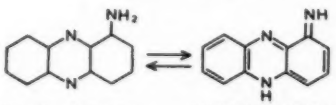
By analogy with 1- and 2-phenazinol, which have been shown to be tautomeric, respectively, with 1(5H)-phenazinone, and 2(10H)-phenazinone[3], it seems highly probable that equivalent systems exist with 1- and 2-aminophenazine:



1-Phenazinol — 1(5H)-Phenazinone or 1,5-dihydro-1-ketophenazine

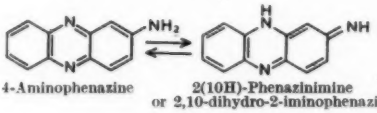


2-Phenazinol — 2(10H)-Phenazinone or 2,10-dihydro-2-ketophenazine



1-Aminophenazine — 1(5H)-Phenazinimine or 1,5-dihydro-1-iminophenazine

II



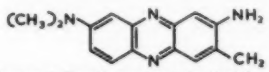
4-Aminophenazine — 2(10H)-Phenazinimine or 2,10-dihydro-2-iminophenazine

III

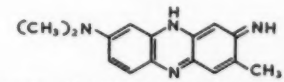
Following the nomenclature of the corresponding keto compounds: it is suggested here that II might be called 1(5H)-phenazinimine, and III, 2(10H)-phenazinimine, as shown above.

Just as Badger, Pearce, and Pettit have stated[3] that the system 1-phenazinol–1(5H)-phenazinone must exist primarily, if not entirely, in the hydroxy form, due to hydrogen bonding with the nitrogen atom in the 10-position, so, too, it is probable that the amino form predominates in the system 1-aminophenazine–2(5H)-phenazinone, even though the predominance would not be expected to be as great. For the sake of academic completeness, however, both the 1-aminophenazine and the 2-aminophenazine systems are illustrated here.

The tautomers of the unsubstituted phenazinol–phenazinone systems apparently are incapable of independent existence, as by analogy are probably those of the unsubstituted aminophenazine–phenazinimine systems also. However, appropriate substitution has made it possible to isolate both forms in one system, at least, that of 1,3,4-trimethyl-2-phenazinol–1,3,4-trimethyl-2(10H)-phenazinone[4]. Hence, in view of the demonstrated fact that separable isomers of 2-aminophenazine do exist when the compound is further substituted by an amino or hydroxyl group in the 8-position[1], it seems a reasonable hypothesis that their structures are wholly analogous to those of the phenazinol–phenazinone systems. If this be true, the two forms of the colour base of neutral red must be as shown below:



2-Amino-8-dimethylamino-3-methylphenazine



8-Dimethylamino-3-methyl-2(10H)-phenazinimine or 2,10-dihydro-8-dimethylamino-2-imino-3-methylphenazine

Existence of a third compound, arising from the higher-temperature neutralization of the sulphuric acid solution of the non-staining form of the base, as is likewise mentioned in the paper by Vivian and Belkin[1], may possibly be due to polymerization under these conditions, but no definite conclusions can be drawn without further work.

DONALD L. VIVIAN

College of Pharmacy,
University of Arizona,
Tucson, Arizona.

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Constraints in the Determination of Active-Centre Topography

THE inactivation of succinic semialdehyde dehydrogenase by trypsin has recently been found to be accelerated by a factor of greater than 100 when triphosphopyridine nucleotide, a substrate of the dehydrogenase, is included in the reaction mixture[1]. Evidence was presented that the site at which triphosphopyridine nucleotide is effective is the same for both dehydrogenase activity and for acceleration of inactivation by trypsin. It was concluded that the binding of a pyridine nucleotide substrate to succinic semialdehyde dehydrogenase produces a configurational change in the enzyme, and, in so doing, results in the exposure of a linkage labile to trypsin. Indirect evidence of such configurational changes has been noted by others[2–4] and suggests that the phenomenon may be of widespread occurrence.

These considerations lead to pertinent deductions concerning the relationship of enzyme topography and enzyme activity. Consider the above-noted case of succinic semialdehyde dehydrogenase[1]. Clearly, the enzyme can exist in at least two different geometric shapes which may be designated as form *A* (the structure of the enzyme in the absence of triphosphopyridine nucleotide) and form *B* (the enzyme in the presence of triphosphopyridine nucleotide), respec-

tively. It is understood that additional forms of the enzyme are not precluded. Since the active centre of an enzyme may be deformed by interaction with the substrate, simultaneous determination of the reaction-rate and the original geometry of the enzyme molecule (form A) are not possible. It should be noted that in actual practice estimations of the dimensions of enzyme active centres are arrived at by measuring relative reaction-rates of substrates and structurally related inhibitors. In view of the foregoing argument, these data cannot logically be used to approximate the molecular configuration of the initial state of the enzyme, that is, form A.

A possible extension of this concept is that the active centre may not be deformed by each substrate in precisely the same manner, but may be shaped within limits by the particular substrate[3,4].

MARSHALL W. NIRENBERG*
WILLIAM B. JAKOBY
National Institutes of Health,
Bethesda,
Maryland.

* Public Health Service Research Fellow of the National Institute of Arthritis and Metabolic Diseases.

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World-wide Distribution of Iodine-131 in Animal Thyroids following Announcements of Isolated Nuclear Weapons Tests in North Africa

Most nuclear weapons tests have been associated with repeated releases of fission products within a few weeks, and the iodine-131 in animal thyroids increased progressively with succeeding tests[1]. In February and April 1960, individual nuclear weapons tests were announced to have occurred in the Sahara Desert. The results on which this report is based were obtained by analysis of preserved thyroid glands sent to the University of Tennessee by volunteer correspondents.

The gamma-radioactivity of the thyroid glands was measured as described in 1956 (ref. 1) and the radioactivity was corrected to the date of slaughter of the animals.

The graphs in Fig. 1 show that iodine-131 of the animal population had reached very low levels before February 10, 1960, except for animals raised near Nashville, Tennessee. The first increases in thyroid iodine-131 content following the test of February 13, 1960, were found in sheep from Haifa, Israel, on February 21, and Portugal on February 23, 1960. On February 24, the cattle from Formosa contained one hundred times more iodine-131 than they previously had in January and February of 1960. There is a strong suggestion that pigs from Memphis, Tennessee, had increased amounts by February 24, 1960. In March 1960, there were increases in thyroid radioiodine in all the animal populations which I sampled. The highest concentrations of radioiodine were found in the thyroids of sheep from Portugal; otherwise the concentrations of radioiodine in animal thyroids reached quantitatively similar maxima in sheep from Germany, London and Nashville and in cattle from Memphis and Formosa. The sheep thyroids generally contained more radioiodine than those from cattle.

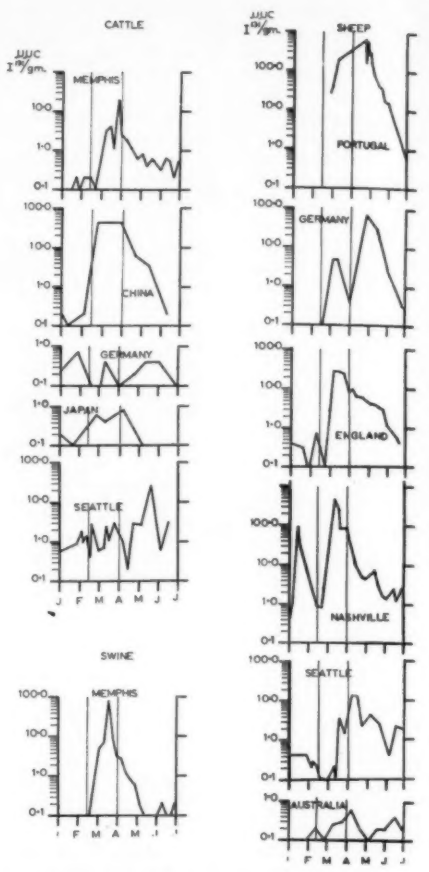

Fig. 1. Micromicrocuries of iodine-131 per gm. of thyroid corrected to date of slaughter of unselected slaughterhouse animals from January to July 1960. 788 thyroids from Memphis cattle, 302 Memphis pigs and 394 Nashville sheep were tested. 83 thyroids from the Republic of China, sent by Dr. Yu-hsi M. Wang, University of Taiwan. 35 cattle and 61 sheep thyroids from Munich, Germany, sent by Karl Schury of Max Muhlpointner A.G. Glands from 464 sheep were sent by E. F. McCleery from London. 35 cattle thyroids were sent from Japan by R. Tanaka, Ibaraki University. 111 cattle and 176 sheep glands were sent by Mr. A. Cirolini, Seattle, Washington. 247 sheep thyroids were sent by Dr. Roger Melick, of the University of Melbourne. 161 sheep thyroids were sent by Dr. J. Amaral Mendes, of the Laboratorio Nacional de Investigacao Veterinaria, Lisbon. Dr. Kurt Steinitz, of Haifa, Israel, sent sheep thyroids slaughtered in Haifa on February 21 and 24, 1960

The relatively large concentration of thyroid iodine-131 in pigs from Memphis was unexpected.

There is a definite tendency towards biphasic iodine-131 concentration curves in Fig. 1 during the period February–April, even though it is thought that only one nuclear test was conducted during that time. This biphasic character is similar to curves obtained from Australian sheep in December of 1957 (ref. 2) and may be characteristic of iodine-131 accumulation after nuclear tests. Another explanation of two maxima between February and April would be the presence, that I cannot explain, of new iodine-131 in the atmosphere, similar to the increased iodine-131 observed in sheep at Nashville in January 1960. After April 1, 1960, in most animal populations the radioactive iodine disappeared with a half-life which was greater than eight days, probably because of the

addition of new fission products after April 1. The sheep population around Munich, Germany, and in Portugal received the most iodine-131 after the test of April 1, but the sheep in London and Nashville received the greatest amounts of iodine-131 before the April 1 nuclear test. The observations available are not sufficient to explain these differences. Cattle in Japan and sheep in Australia showed very small amounts of iodine-131 from the weapons test in North Africa. The amount of fall-out in Australia was probably reduced by the great distance of Melbourne from the latitude of origin of the fission products. Japanese cattle have usually had less iodine-131 than other populations during the past five years.

The maximum concentration of iodine-131 in the sheep at Nashville was observed in March and quantitatively it was similar to that observed 38 months previously, when the iodine-131 in sheep thyroids (in December 1956) was believed to have had its origin in northern Siberia in November 1956.

These observations show again that the monitoring of radioiodine in thyroid glands of grazing animals is a sensitive and informative method for evaluating the biological exposure of animal populations to fission products.

This investigation was supported by a research grant from the United States Atomic Energy Commission.

L. Van Middlesworth

Department of Physiology,
University of Tennessee,
Memphis,
Tennessee.

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PHYSIOLOGY

Auto-Antibodies against Antigen derived from Colon in the Microsomes of Regional Colonic Lymph Glands in Human Ulcerative Colitis

The sera of children with ulcerative colitis have been shown to contain a γ-globulin which reacts with an antigen prepared by phenol–water extraction of human colon. The serum factor may be termed an auto-antibody[1]. This communication presents evidence that antibody against the same antigen also occurs in the regional colonic lymph glands but not in the ileal glands of the patients and that it is bound to the microsomes of the colonic glands.

About 10 gm. of regional colonic lymph glands were obtained during colectomy from each of two children (*A* and *B*) with severe ulcerative colitis. In patient *B* regional ileal lymph glands (5 gm.) were obtained from the mesentery at a distance of 40 cm. from the ileocæcal valve. Serum from these two patients and from others was also available.

Immediately after surgery the glands were homogenized with a Potter–Elvehjem homogenizer in ice-cold 0·25 *M* sucrose with 0·001 *M* Mg^{2+}. Twice-washed microsomes (sediment after centrifugation at 105,000*g*) and cell sap (final supernatant) were prepared following Kern *et al.*[2]. The microsomal sediments were finally homogenized again and suspended in the sucrose medium to the original concentration. Protein was determined according to ref. 3.

Table 1. Hæmagglutination of Sheep Red Blood Cells coated with Phenol–Water extracted Antigen from Human Colon

Test solutions	Patient *A*		Patient *B*	
	Protein (mgm./ml.)	Titre*	Protein (mgm./ml.)	Titre*
Microsomes from colonic glands	3·75	0	4·00	0
Cell sap from colonic glands	10·50	16	13·50	64
Microsomes from ileal glands			4·50	0
Cell sap from ileal glands			16·00	0
Serum of patient		128		32
Norman human serum		0		0
Physiological saline		0		0

* The titres are the reciprocal of the highest dilutions giving macroscopically visible hæmagglutination when 1 ml. aliquots of serial dilutions of the fractions or the sera were mixed with equal volumes of a 0·5 per cent suspension of coated red cells. The antigen solution used for coating contained 100 μgm./ml. of the lyophilized material. The two experiments (patients *A* and *B*, respectively) were made on different days, using different samples of red blood cells and different antigen solutions for coating. Controls with uncoated red blood cells were negative in all cases.

In the immunological tests, sheep red blood cells were coated by the Middlebrook–Dubos method[4] with antigen extracted from fœtal colon[1]. Cell sap and sera were inactivated (56° C., 20 min.) before use and absorbed with sheep red cells to remove heterophile antibodies.

Table 1 shows the results of two typical experiments. As can be seen, the hæmagglutinating activity was confined, in both patients, to the serum and cell sap of the colonic lymph glands. The cell sap of the ileal lymph glands was inactive. No hæmagglutination was obtained with any of the three microsomal fractions. However, there is some evidence that antibody bound to microsomes is non-hæmagglutinating[5]. On the other hand, Kern *et al.*[2] have demonstrated antibody in microsomes of lymph nodes from immunized animals by its antigen-combining capacity. Therefore, in a second series of experiments, the antigen-binding capacity of serum and of the various fractions from the colonic lymph

Table 2. Hæmagglutination of Sheep Red Blood Cells, coated with Antigen incubated with Various Preparations from Patient *A**

Incubations	1 antigen + microsomes	2 antigen + cell sap	3 antigen + antiserum *A*	4 antigen + normal serum	5 antigen + saline
	Incubation† Coating‡ of red blood cells Hæmagglutination§				
Titres	0	0	0	64	64

* See Table 1 for analytical data.

† 1 ml. aliquots of antigen in saline (150 μgm./ml.) were incubated for 4 hr. at 20° C., with 2 ml. of either one of the samples indicated in columns 1–5. Columns 4 and 5: controls. No precipitate appeared in the incubation mixtures.

‡ 1 ml. of a red blood cell suspension was added to each of the incubation mixtures.

§ After washing, hæmagglutination was performed with a known hæmagglutinating serum from another patient. Titres are the reciprocals of the highest dilution of antiserum giving macroscopically visible hæmagglutination with equal volumes of a 0·5 per cent suspension of coated red cells.

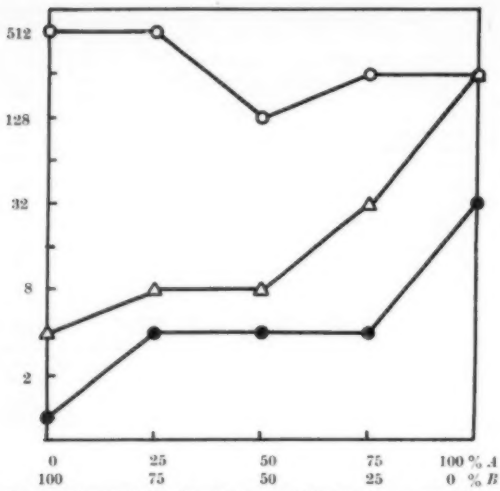

Fig. 1. Hæmagglutination of sheep red blood cells, coated with antigen incubated with various preparations from patient *B*. 1 ml. aliquots of the antigen (150 μgm./ml.) were incubated with 2 ml. of either undiluted microsomes or cell sap (see Table 1 for analytical data), serum from the patient, or normal serum. In addition, aliquots of the antigen were also incubated with 2 ml. samples of microsomes, diluted with their homologous cell sap in three different proportions, or with similar mixtures of patient serum and normal serum. For details of incubation, see Table 2. The hæmagglutinations were performed with a known positive serum from another patient. Abscissa, proportions (per cent) in which the various fractions (*A* and *B*) were mixed before they were added to the antigen for incubation. Ordinate, reciprocal of highest dilution of antiserum giving macroscopically visible hæmagglutination. In the control where antigen had been diluted with 2 ml. of physiological saline, the hæmagglutination end-point was 512.
●, Colonic glands; *A*, cell sap; *B*, microsomes. ○, Ileal glands; *A*, cell sap; *B*, microsomes. △, Serum; *A*, normal serum; *B*, antiserum

glands was measured. The procedure is outlined in Table 2, which shows the results of a typical experiment with the specimens from patient *A*. It will be seen that incubation of the antigen with normal human serum did not abolish its ability to sensitize the red cells. In contrast, incubation of the antigen with microsomes, cell sap or serum of the patient prevented hæmagglutination completely. Similar results were obtained with the specimens from the second patient. Fig. 1 shows the typical results of an experiment made with all the samples obtained from patient *B*. The figure reveals that the inhibiting capacity of the cell sap from the colonic glands was appreciably lower than that of an equally diluted sample of the microsomes. The inhibitory capacity of undiluted serum from this patient was also lower than that of the colonic microsomes. However, since the chemical nature of the antibodies is unknown, no conclusions can be drawn regarding their concentration in the different preparations. Microsomes and cell sap from the ileal glands, as well as normal human serum, were inactive.

The mechanism of hæmagglutination inhibition by immune microsomes is unknown. It could be assumed that both antibody-active particles and antigen are bound to the red cells. In this case the molecular structures responsible for adsorption of the antigen to the red cells would be different from the structures combining with antibody. This has actually been shown for the α-hæmosensitisin of *Mycobacterium tuberculosis*[6]; this substance can be adsorbed directly to the red blood cells by the Middlebrook–Dubos procedure[4]. Although the immunological activity resides in the polysaccharides constituting the bulk of the material, a small lipid fraction seems to be necessary for sensitization[6].

The finding of antibodies in colonic gland microsomes is in agreement with that of other workers who found antibodies in splenic or lymphatic microsomes of immunized animals[2,5,7]. In contrast, microsomes (and cell sap) of more distant lymph glands were devoid of antibody activity. This would appear to rule out the possibility that the antibodies in the regional colonic glands were adsorbed serum antibodies. In this connexion, it should be mentioned that Kern *et al.*[2] could not find any adsorption of antibodies when antiserum was added to isolated microsomal fractions from non-immunized animals. Rather, then, the presence of antibodies in our samples would reflect a synthetic activity occurring in the microsomes of certain cells in the regional colonic glands. There is considerable evidence that proteins which are secreted are synthesized by the ribonucleoprotein particles, constituting a part of the microsomal fraction[8,9]. Before being released into the cell sap or the medium, the products of the synthetic activities of the particles often remain temporarily attached to the membranous elements which also are obtained in the microsomal fraction upon ultracentrifugation[8]. In order to study the mode of intracellular binding, attempts were made to solubilize antibodies from the microsomes by means of phospholipases and various detergents which are known to liberate proteins from the membranous parts of the microsomal fraction[8,10,11]. In a number of preliminary experiments, positive precipitin reactions were obtained in agar diffusion tests, using extracts of microsomes treated with phospholipase *A* from bee venom. Preliminary attempts to liberate precipitating or hæmagglutinating antibodies from isolated ribonucleoprotein particles by increasing the *p*H, treatment with chelating agents or with ribonuclease[5,7] have been unsuccessful.

In the homograft reaction, immunologically competent cells have been shown to be localized in the regional lymph nodes[12]. If immune reactions play any part in the pathogenesis of ulcerative colitis, it is likely that the lesions are the expression of a hypersensitive state, mediated by cell-bound antibodies of the same type as those participating in the homograft reaction, or in delayed hypersensitivity[12]. It is therefore of interest that, in the present case, isolated subcellular structures of regional colonic lymph glands exhibit an antigen-binding activity without foregoing extraction. Thus tissue lesions might be initiated by the binding of antigen on cells of the colon to antibody-bearing membranes of the immunologically competent cells. It is possible that in the latter cells antibody-active protein is transported from its site of formation, perhaps on the ribonucleoprotein particles, to the surface of the cell. The cell membrane is supposed to be chemically and morphologically related to the endoplasmic reticulum and its fragments will probably appear in the microsomal fraction[14]. A surface antibody of this nature could be of some importance in delayed hypersensitivity[15].

This work was supported by a grant from the Swedish Medical Research Council. We are indebted to Drs. Th. Ehrenpreis and N. Ericsson (Surgical Department of the Pediatric Clinics, Karolinska Sjukhuset, Stockholm), who have kindly provided the

material, and to Dr. G. Asherson, Taplow, for reading the manuscript and helpful suggestions.

PETER PERLMANN
OVE BROBERGER

Wenner-Gren Institute for
Experimental Biology,
University of Stockholm,
and the
Pædiatric Clinic of the
University of Gothenburg.

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Diurnal Changes in the Topographical Distribution of Glycogen in the Brain of *Iguana iguana*

RECENTLY I investigated the topographical distribution of glycogen in the brain of *Iguana iguana* in relation to the environmental temperature, and in animals with glycogen-accumulation disease[1]. It appeared that a decrease in environmental temperature results sometimes in a decrease in concentration of the glycogen in the brain tissue, and in some cases an increase, for in animals with heptomegalia glycogenica the glycogen concentration in the tissue of the cortex cerebri, the diencephalon, the mesencephalon, the cerebellum and the medulla oblongata increases significantly.

In continuation of these experiments the diurnal changes in the brain glycogen of *Iguana* were investigated. For this purpose the concentration of the brain glycogen was determined in 60 males and 60 females at 6-hr. intervals in the five topographical regions of the brain mentioned. The environmental temperature at which the animals were kept was $36 \cdot 8 \pm 0 \cdot 3°$ C. For the glycogen determination I used Kerr's method[2], the reduction compounds being determined by Nelson's method[3] (see also in this connexion the paper of Jakoubek and Svorad[4]). The glycogen values are given in mgm./100 gm. fresh brain tissue in Table 1. For the determination of each value 15 animals were used.

As can be seen from Table 1, there are no significant periodic changes in the cortex cerebri, the mesencephalon, the cerebellum and the medulla oblongata. However, in the diencephalon a significant difference in the glycogen concentration can be seen.

Thus, the changes in concentration of the brain glycogen not only run parallel to several altered states of behaviour in the individual[6,7], but are also related to the autonomic functions, which are regulated at the highest level.

These results correspond with those of Svorad[5] obtained in the rat.

A. STOLK

Histological Laboratory,
Free University,
Amsterdam.

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Muscular Dystrophy in the Siamese Fighting Fish as an Aberration of the Connective Tissue

RECENTLY I carried out a histochemical study of the ability of connective tissue in normal muscles and atrophied muscles of *Iguana iguana* to dephosphorylate various high-energy phosphates derived from adenosine monophosphate and some other compounds[1].

In continuation of this work I carried out a corresponding investigation in a fish, namely, the Siamese fighting fish *Betta splendens* Regan, belonging

Table 1. DIURNAL CHANGES IN GLYCOGEN CONCENTRATION IN THE BRAIN OF *Iguana iguana*. THE GLYCOGEN VALUES ARE GIVEN IN MGM./100 GM. FRESH BRAIN TISSUE. FOR THE DETERMINATION OF EACH VALUE 15 ANIMALS WERE USED. M, MALE ; F, FEMALE

Parts of the brain	Sex	Astronomical time 1 hr.	7 hr.	13 hr.	19 hr.
Cortex cerebri	m	$88 \cdot 1 \pm 3 \cdot 9$	$84 \cdot 6 \pm 6 \cdot 0$	$86 \cdot 3 \pm 4 \cdot 6$	$87 \cdot 2 \pm 4 \cdot 7$
	f	$86 \cdot 9 \pm 5 \cdot 4$	$83 \cdot 9 \pm 4 \cdot 1$	$85 \cdot 8 \pm 5 \cdot 1$	$85 \cdot 9 \pm 3 \cdot 9$
Diencephalon	m	$104 \cdot 9 \pm 5 \cdot 7$	$121 \cdot 9 \pm 4 \cdot 9$	$124 \cdot 7 \pm 6 \cdot 0$	$114 \cdot 9 \pm 5 \cdot 8$
	f	$107 \cdot 2 \pm 4 \cdot 0$	$123 \cdot 6 \pm 5 \cdot 7$	$126 \cdot 1 \pm 4 \cdot 8$	$117 \cdot 2 \pm 4 \cdot 9$
Mesencephalon	m	$117 \cdot 1 \pm 5 \cdot 1$	$111 \cdot 8 \pm 4 \cdot 7$	$114 \cdot 2 \pm 7 \cdot 1$	$115 \cdot 3 \pm 6 \cdot 0$
	f	$116 \cdot 3 \pm 4 \cdot 3$	$110 \cdot 7 \pm 4 \cdot 3$	$113 \cdot 1 \pm 6 \cdot 0$	$114 \cdot 8 \pm 5 \cdot 5$
Cerebellum	m	$80 \cdot 9 \pm 5 \cdot 9$	$78 \cdot 1 \pm 6 \cdot 0$	$80 \cdot 7 \pm 5 \cdot 1$	$81 \cdot 4 \pm 5 \cdot 9$
	f	$82 \cdot 3 \pm 5 \cdot 6$	$79 \cdot 8 \pm 4 \cdot 5$	$82 \cdot 5 \pm 4 \cdot 2$	$83 \cdot 1 \pm 5 \cdot 3$
Medulla oblongata	m	$112 \cdot 0 \pm 4 \cdot 7$	$107 \cdot 5 \pm 5 \cdot 6$	$109 \cdot 3 \pm 4 \cdot 7$	$109 \cdot 0 \pm 4 \cdot 2$
	f	$113 \cdot 4 \pm 5 \cdot 7$	$110 \cdot 1 \pm 3 \cdot 8$	$111 \cdot 6 \pm 5 \cdot 3$	$110 \cdot 2 \pm 5 \cdot 8$

to the family Anabantidae. In this species a distinct senile period occurs, in which the animals are seen to move slowly near the surface of the water. For this investigation very old specimens were used, in which a progressive muscular dystrophy had occurred.

For the determination of the 5-nucleotidase activity of the material, the histochemical methods of Beckett and Bourne[2] were used.

Since adenosine monophosphate is dephosphorylated by 5-nucleotidase, the ability of the connective tissue to bring about dephosphorylation of various high-energy phosphates derived from adenosine monophosphate in the normal and atrophied muscles of *Betta* was investigated. In addition, some other phosphate compounds of a certain physiological importance and some other esters were investigated. These substances were adenosine diphosphate, adenosine triphosphate, creatine phosphate, acetyl phosphate, cytidine, inosine, guanosine, pyridoxal phosphate, thiamine pyrophosphate, riboflavin-5-phosphate, diphosphopyridine nucleotide, triphosphopyridine nucleotide, phosphoglyceraldehyde, hexosediphosphate, fructose-1- and 6-phosphates and glucose-1- and glucose-6-phosphates. The histochemical reactions of all these compounds were compared with that of β-glycerophosphate.

In the 81 cases of muscular dystrophy in *Betta*, with the exception of creatine phosphate, dephosphorylation of the above-mentioned high-energy phosphate compounds by the elements of the connective tissue and by a number of the muscle fibres was observed. However, the intermediate glycolytic compounds showed no hydrolysation.

By the dephosphorylation experiments in fourteen of the 81 cases I was able to establish a dephosphorylation of thiamine pyrophosphate. The co-enzymes pyridoxal phosphate and riboflavin-5-phosphate could not be demonstrated to be affected.

In all cases of progressive muscular dystrophy in *Betta* the elements of the connective tissue were found to dephosphorylate the diphosphopyridine nucleotide and the triphosphopyridine nucleotide. Just as in the muscular dystrophy of man[3] and of *Iguana*[1], in the case of the diphosphopyridine nucleotide a stronger dephosphorylation was observed than in that of the triphosphopyridine nucleotide.

Summarizing, we may conclude that in the elements of the connective tissue surrounding the muscle fibres, enzymes destructive of compounds vital to muscular metabolic processes have been identified with certainty. These enzymes must no doubt play a certain part in the genesis of the progressive muscular dystrophy.

Just as is the case in man[3] and in *Iguana*[1], the pathological changes in the muscles of *Betta* in progressive muscular dystrophy consist of an increase in number of the sarcolemmal nuclei, an atrophy of the muscle fibres, a great diversity of the size of the muscle fibres, a proliferation of the elements of the connective tissue and storage of varying amounts of fat. The sarcolemmal nuclei may assume the form of chains localized centrally in the muscle fibres. Between these changes and those in progressive muscular dystrophy in man there is a good correspondence.

Adams, Denny-Brown and Pearson[4] arrived at the conclusion that phagocytosis of degenerating muscle fibres is rare. Bourne and Golarz[3], however, found evidence in a number of cases for what appears to be an invasion and a destruction of muscle fibres by the neighbouring connective tissue.

Bourne and Golarz[3], who by their study of ten cases of progressive muscular dystrophy in man obtained corresponding results, have given three possible interpretations, namely: (1) The primary defect may still be in the muscle-fibre membrane. In this connexion, the diffusion of the phosphate esters from the muscle fibres into the neighbouring medium may result in the adaptive synthesis of certain enzymes to hydrolyse them in the elements of the connective tissue. (2) The synthesis of the metabolically very important phosphate compounds occurs in the muscle fibres, but nevertheless there may be some leakage in the normal metabolism, much in the same way as the vitamin A leaks away from the retina in the retinene-cycle. The replacement of this leakage by transport from the lymphatic and the vascular system, if there is inadequate synthesis by the muscle fibres themselves, would be prevented by the connective tissue elements, which contain the appropriate phosphorolytic enzymes. (3) The production of the hydrolytic enzymes by the elements of the connective tissue may lead to diffusion of these enzymes into, as well as adsorption on to, the membrane of the muscle fibres, and possibly penetration of this membrane, resulting in a complete blockage of the normal metabolic processes of these fibres.

Comparing these possible interpretations, Bourne and Golarz[3] came to the conclusion that the fundamental defect in progressive muscular dystrophy in man may be in the elements of the connective tissue supporting the muscle fibres, and not in these fibres themselves (possibility 1).

On the other hand, possibility 2 is not sufficiently in agreement with current views on the metabolic processes in the muscle fibre. Possibility 3 is not so probable either, because the penetration of the sarcolemma by the hydrolytic enzymes has still to be established. It is not improbable that the accumulation of such a range of phosphatases at this site might break down its structural integrity as well as its selective permeability.

My results obtained in the muscular dystrophy of *Betta* and of *Iguana*[1] point in exactly the same direction, and may therefore be an argument for the concept that an aberration of the neighbouring connective tissue is the primary cause of the muscular dystrophy in fishes as well as reptiles.

A. Stolk

Histological Laboratory,
Free University,
Amsterdam.

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Initiation of Mammary Secretion in Rats by Electrical Stimulation of Nervous System

During recent years the interaction of neural and endocrine factors in the initiation of mammary secretion in different species of animals is being increasingly recognized, and it has been shown that electrical stimulation of the uterine cervix of œstrogen-primed virgin female rats can initiate mammary

secretion[1]. In the present experiments, attempts were made to determine whether electrical stimulation of other areas would similarly result in mammary secretion.

Virgin female rats were injected subcutaneously daily with 10 μgm. œstradiol for 10 days to develop their mammary glands. For the following 5 days, groups of rats were electrically stimulated for 30 sec. twice daily in the regions of the head, lumbar vertebræ, nasal mucosa and nipples. Control rats were injected twice daily with saline for 5 days. On the sixteenth day, the rats were killed, and the right inguinal mammary glands were removed for histological preparations. The mammary glands of the controls regressed to a bare duct system, whereas almost all the electrically stimulated rats showed maintenance of the mammary lobule-alveolar system and initiation of lactation. These results suggest that electrical stimulation of the central nervous system induces release of prolactin and adrenocorticotrophic hormone from the anterior pituitary, since both hormones are required to induce mammary secretion in the œstrogen-primed rats.

M. Maqsood
J. Meites

Department of Physiology and Pharmacology,
Michigan State University,
East Lansing.

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HÆMATOLOGY

Anti-i : a New Cold Antibody

In 1956 Wiener and his colleagues[1] described an antibody occurring in a case of cold antibody hæmolytic anæmia which they called anti-I. The I antigen has an almost universal distribution, but five examples of i cells were found in testing 22,000 donors. It was considered that the Ii phenotypes arose by the action of two allelomorphic genes *I* and *i*, individuals of the phenotype i being *ii*.

More recently, Jenkins *et al.*[2] have described an English donor whose cells are i and whose serum contains a naturally occurring anti-I that is not auto. Using these cells, the antibodies in a number of other cases of cold antibody hæmolytic anæmia were also found to have I specificity. At the same time Tippett *et al.*[3] have reported a number of blood samples that are extremely weak in I although not as weak as the i donor of Jenkins *et al.* The serum of these people also contains anti-I that is not auto.

The results of the above workers show clearly that i cells also contain some I, but an enormous gulf separates them from the I of normal adults. Cord-blood cells were also found to be extremely weak in I.

Recently, we encountered a remarkable antibody that appears to clarify the Ii relationship in human red cells. This antibody was found in the serum of a patient not yet diagnosed but probably suffering from a reticulosis.

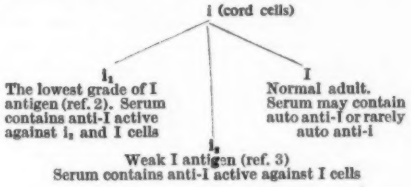
The serum of this patient weakly agglutinated his own and 500 normal adult red cell samples at low temperature (average titre at 12° C. about 1 in 16). Tests with three examples of i cells and five examples of the slightly stronger variant described by Tippett *et al.* all gave strongly positive results with titres ranging from 1 in 32,000 up to 1 in 250,000, the three i samples all giving maximum titres. More than 40 cord blood samples were tested and all gave strong reactions similar to adult cells of the i phenotype.

Repeated absorptions of the serum with various cells showed that a single antibody was present reacting powerfully with i and cord cells and weakly with adult I cells. Clearly this antibody has anti-i specificity. Electrophoretic studies have shown that the antibody is present in the β-globulin fraction of the serum.

Tests on a number of infants of various ages show that within 18 months of birth the i reaction becomes steadily weaker while the I reaction becomes stronger until the normal adult relationship of strong I and weak i is established. It appears that all normal adult and infant samples have some I and some i, and cells that are strong in I are weak in i and vice versa.

It is clear that the abnormality found in the cells of the rare adults of the i phenotype is not that they are i, for everyone is probably i for a few weeks of life, but that the I has failed to develop. This type of reaction surely cannot reflect the action of *I* and *i* as allelomorphic genes. We think that the abnormality found in these i adults is caused by the absence of a genetically controlled factor that is essential for the proper development of I.

Our results have shown that the anti-i serum is able to place people very precisely on the I scale in an indirect way by measuring the amount of i that they possess. In view of this the following terminology in the I group system is suggested :


i (cord cells)
I_1
The lowest grade of I antigen (ref. 2). Serum contains anti-I active against I_1 and I cells
I
Normal adult. Serum may contain auto anti-I or rarely auto anti-i
I_2
Weak I antigen (ref. 3)
Serum contains anti-I active against I cells

Details of these results will be submitted for publication elsewhere.

W. L. Marsh
W. J. Jenkins

North East Metropolitan
Regional Blood Transfusion Centre,
Brentwood,
Essex.

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Ageing of Hæmoglobin

The problem of ageing of protein molecules has been considered by several authors. Kunkel[1] in man, Ranney[2] in the rabbit, have attempted to demonstrate the ageing of hæmoglobin, taking advantage of the metabolic heterogeneity of hæmoglobins described by us in 1950[3]. They observe, indeed, during the life of the red cells, an equalization of the specific activities of hæmoglobin fractions which were unequal initially. These authors were using iron-59, and it is known that iron is re-utilized after the death of the red cells : this prevents observation of an eventual crossing of the specific activity curves of different fractions in the course of the ageing of the

red cells. Therefore they cannot decide between the two following hypotheses: (1) there are metabolic exchanges between the fractions, contrary to the theory of the metabolic inertness of hæmoglobin; (2) there is ageing of part of the hæmoglobin, which during this process changes its electric charge and becomes concentrated in one of the fractions.

In an attempt to overcome this difficulty, we used valine labelled with carbon-14, which we injected intraperitoneally into rabbits weighing 2–3 kgm. at a dosage of 8 μc./kgm. Blood was removed at various intervals from 1 to 80 days after injection.

Hæmoglobin was prepared as the carbon monoxy derivative and purified by salting out[4]. Checks of purity were made by starch-gel electrophoresis in phosphate buffer *p*H 6·5, μ 0·033 (ref. 5). The hæmoglobin samples were then submitted to electrophoresis on starch blocks in veronal buffer *p*H 8·6, 0·05 *M* for 16 hr. under 5 V./cm.

Under such conditions we obtained two fractions: a major one (R_1) and a minor one which migrated more towards the anode (R_3). No fraction corresponding to hæmoglobin A_2 was observed in the rabbit. The respective amounts of main and minor fractions were 90 and 10 per cent. These percentages were relatively constant from one rabbit to the other and did not vary for the same rabbit.

Each fraction was eluted with water and centrifuged at 15,000*g* for 1 hr. The eluates were precipitated with trichloracetic acid, washed several times with water, acetone and ether, and dried *in vacuo*. The powders so obtained were prepared as pellets (compression, 5,000 lb. per sq. in.). Their activity was then determined with a flow counter and the specific activity computed after correction for thickness.

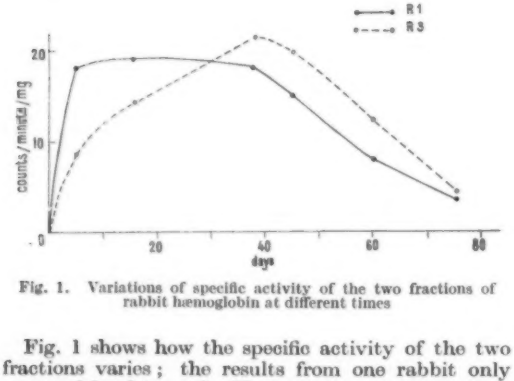

Fig. 1. Variations of specific activity of the two fractions of rabbit hæmoglobin at different times

Fig. 1 shows how the specific activity of the two fractions varies; the results from one rabbit only was used for this graph. The experiment was repeated on six different rabbits, and results are given in Table 1.

The specific activity of R_1 increases up to a plateau, which is reached after 5 days and is maintained for about 40 days, after which it declines. That of R_3 is initially lower; it reaches and passes that of R_1 near the thirty-fifth day and then begins to decrease without showing a plateau, but it remains higher than R_1.

These observations confirm once more the metabolic heterogeneity of rabbit hæmoglobin. On the other hand, the inversion of the ratio of the specific activity of the hæmoglobin fractions according to time is a completely new fact. It involves a progressive enrichment in ^{14}C valine of the R_3 fraction with time.

Table 1. Ratio of Specific Activity, R_3/R_1

	Days after the injection	Ratio
Rabbit No. 1	5	0·60
	16	0·75
	37	1·18
	45	1·30
	60	1·30
	80	1·20
Rabbit No. 2	6	0·70
	23	0·70
	50	1·20
	72	1·00
Rabbit No. 3	5	0·67
	39	1·00
	55	1·40
Rabbit No. 4	1	0·32
	4	0·52
	46	1·25
	60	1·20
Rabbit No. 5	27	0·75
	46	1·30
Rabbit No. 6	21	0·75
	23	0·82
	52	1·28
	59	1·18

This fact cannot be explained only by metabolic exchanges between the two hæmoglobin fractions, which could at most give rise to an equalization of the specific activities. The most likely explanation is that, during the ageing of the red cell, part of the hæmoglobin itself undergoes modification. It becomes more electronegative, which changes its motility. So after the fortieth day the anodic fraction contains a high proportion of the labelled hæmoglobin. Its specific activity becomes greater than that of the main cathodic fraction, the radioactivity of which has been diluted by a large amount of newly synthesized, and therefore non-labelled, hæmoglobin. These modifications must be distinguished from those observed on blood aged *in vitro*[6], but their nature may be the same. Electrophoretic modifications of hæmoglobin have been described[7] in red cells aged *in vivo*; but these modifications may be due to difference in the molecular environment in the red cell with ageing. Our observations lead to the assumption of a change of hæmoglobin configuration, involving exteriorization of negative polar groups or, on the other hand, dissimulation of positive polar groups.

This work was supported by grants from La Caisse Nationale de Sécurité Sociale (France), L'Institut National d'Hygiène (France), Le Commissariat à l'Energie Atomique (France), and by research grants No. *A*–2773 from the National Institute of Arthritis and Metabolic Diseases, No. *RG*–6016 from the Division of General Medical Sciences, U.S. Public Health Service.

Jean Rosa
Jean-Claude Dreyfus
Georges Schapira

Laboratoire de Recherches de Biochimie Médicale,
Hôpital des Enfants Malades,
Paris.

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PATHOLOGY

An Effect of Early Dilution on the Establishment of Lysogeny in *Salmonella typhimurium*

It is known that the frequency of lysogenic response in a culture of *Salmonella typhimurium* strain *LT2*, infected with the temperate bacteriophage *P22*, can be influenced by the ratio of phage to bacterium (multiplicity of infection)[1–3], and by modifying the metabolism of the infected cells soon after infection, for example, by the addition of chloramphenicol[4]. With a high multiplicity of infection a high frequency of lysogenic response is obtained. Using a low multiplicity of infection a low lysogenic response results. If these infected cells are exposed to chloramphenicol (25 μgm./ml.) 5 min. after infection and the chloramphenicol removed by dilution 15 min. later, then practically every cell infected becomes lysogenic.

We have been examining the effect of lower concentrations of chloramphenicol in contact with cells infected with a low multiplicity of infection (2·8) for a shorter period of time. Fig. 1 shows that a concentration of 5 μgm./ml. in contact with the cells for 7 min. is sufficient to reverse the 'decision' of the majority of the cells to lyse. What is more interesting is that the infected control culture shows a high frequency of survivors when sampled and diluted into buffer 5 min after infection. This high frequency of survivors decreases with time until it reaches a minimum at 12 min. In all our previous experiments the control value for lysogenic response was measured at the end of the experiment and thus gave this minimum value.

In view of these results, measurements were made of the frequency of lysogenic and lytic responses of a similarly infected culture when samples were removed and diluted into buffer at room temperature during the first 15 min. after infection. The results are shown in Fig. 2. There is an evolution of the lysogenic response during this period rising to a maximum 6 min. after infection, decreasing to a minimum at 12 min. The lytic response complements the lysogenic response. These results were later found to be true, even when dilutions were made into broth or buffer at room temperature (26·5° C.) or 37° C.

Under the conditions of the experiments above, 80 per cent of the cells would have been infected with at least one phage particle by 2 min. At 6 min., 95 per cent would be infected. This may account for the rise in lysogenic response between 2 and 6 min. after infection.

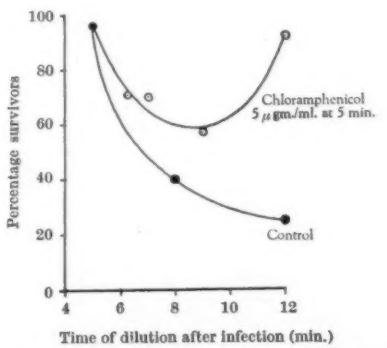

Fig. 1. Effect of dilution on survival of *LT2* cells infected with phage *P22*, with and without chloramphenicol

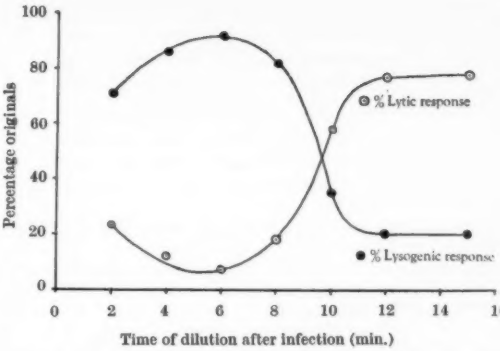

Fig. 2. Effect of dilution on lysogenic and lytic responses in *LT2* cells infected with phage *P22*

Chloramphenicol is said to reverse the 'decision' of a cell to lyse by virtue of its ability to inhibit protein synthesis[4]. The results reported in this communication indicate that dilution into fresh medium is equally effective if carried out before the 'decision' period is reached. This 'decision' period was shown by Lwoff to be 6–10 min. after infection in *S. typhimurium* infected with an *A1* phage[2]. The importance of dilution in a lytic phage system has been reported by Lanni[5], who showed that in the *T5-E. coli F* system dilution of high bacterial concentrations is necessary to stabilize the phage–host complex.

It would be interesting to know whether the effect of early dilution reported here has been found in other lysogenic systems or is restricted to *S. typhimurium*.

B. E. B. Moseley
R. H. Gorrill

Department of Bacteriology,
Guy's Hospital Medical School,
London Bridge, S.E.1.

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A Filterable Hæmolysin from *Escherichia coli*

Some strains of *Escherichia coli* are hæmolytic when grown on blood-agar, and attempts made to demonstrate a soluble hæmolysin in culture filtrates are usually unsuccessful. We have been able to obtain hæmolytic filtrates with four such strains when grown in a culture medium prepared as follows.

A pound of lean beef is cut into small pieces and boiled in 500 ml. of *N*/20 aqueous sodium hydroxide. After simmering for 15 min. it is strained through a muslin cloth, and excess fluid removed. The meat is dried by pressing between filter paper and placed in a flask to which 500 ml. of infusion broth is added; the *p*H is adjusted to 7·6. The medium is sterilized by steaming for 24 hr. and autoclaving for 20 min. at 120° C.

Three of the strains used (0138 : *K81B*, 0139 : *K82B*, and 0141 : *K85B*) were recovered from diseased

pigs[1] and the fourth (*RVC*58—the antigens of which are not yet determined) from diseased calves[2]. The cultures were incubated at 37° C. for 2, 4, 6, 8, 10, 12, 18, 24, 48 and 96 hr., and filtered through 'Ford *SD* Sterimats' at a negative pressure of about 150 mm. of mercury. The filtrates were collected and diluted with physiological saline; they were tested against 1 per cent (final concentration) washed rabbit red cells at 37° C. Examinations were made at half-hourly intervals.

Hæmolytic activity occurred with filtrates from cultures 4–24 hr. old, the maximum activity being with cultures of 6–10 hr. The titre rarely exceeded 1 : 32, and no filtrate prepared from cultures grown for 48 or 96 hr. was hæmolytic. There was little variation in the *p*H of these filtrates (about 7·4–7·6) and all were sterile. The first portion of the fractions passing the filter was always non-hæmolytic, due probably to some absorption by the filter pad.

The hæmolysin is thermo-labile. Filtrates from 6-hr. cultures lost about half their activity when stored at 4° C. for six days, and when left overnight on the bench. Activity was reduced after 18 hr. at 37° C., while it was destroyed after 15 min. at 56° C.

Cultures were incubated in atmospheres of carbon dioxide and of hydrogen, and filtrates from these were also hæmolytic but less active. There was no evidence of a hæmolysin in filtrates from cultures grown in 1 per cent peptone water, infusion broth, 2 per cent, 6 per cent and 10 per cent serum broth.

The hæmolytic activity could be removed by treatment of the filtrate with 30 per cent aqueous ammonium sulphate. The precipitate, when redissolved, dialysed and made isotonic, was less active than the original filtrate and this decrease may be associated with the labile nature of the hæmolysin. Similar precipitates prepared from uninoculated media and a filtrate from a 6-hr. culture of a non-hæmolytic strain were non-hæmolytic.

According to Kunicki-Goldfinger *et al.*[3], hæmolysis induced by strains of *E. coli* is due to the products of autolysis. Our findings are not in accord with this, since hæmolytic activity appears to be confined to young cultures. After 10 hr. incubation this activity declines.

R. Lovell

Department of Pathology,
Royal Veterinary College,
London.

T. A. Rees

St. George's Hospital Medical School,
London.

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Development of a Leukæmia resistant to Cyclophosphamide ('Cytoxan')

The alkylating agent, N,N-bis-(β-chlorethyl)-N′, O-propylenephosphoric acid ester diamide[1] (*B*-518, *cyclo*phosphamide, 'Endoxan', 'Cytoxan'), has been markedly effective in suppressing the growth of a variety of transplanted tumours in rats[2,3] and mice[3]. The mechanism of activation of this agent has not been determined. It is known that the drug is not cytotoxic *in vitro*, but is activated *in vivo*[4]. Nicotinamide has been reported to prevent the toxic and antitumour effects of 'Cytoxan'[5]. However, the relationship of nicotinamide or diphosphopyridine nucleotide to either the activation or the action of this alkylating agent is unknown. Resistant tumours have proved valuable for the study of the mechanism of action of chemotherapeutic compounds. Sublines resistant to alkylating agents and derived from previously sensitive tumours are uncommon and have not been investigated extensively. It was thought that if a tumour resistant to 'Cytoxan' could be developed, it might provide a useful model for the study of the action of this alkylating agent.

The *L*-1210 leukæmia was selected for study because it was sensitive to 'Cytoxan'[6], it could be carried in the ascites form, and it has been employed by others in studies of resistance to various antitumour agents. It has been reported that the effectiveness of 'Cytoxan' in increasing the median survival of mice with advanced *L*-1210 leukæmia was markedly enhanced by a schedule of once-weekly drug administration compared to more frequent treatment[7]. This finding suggested that the isolation of a resistant variant might be facilitated by the use of large single doses of 'Cytoxan'.

Sensitive *L*-1210 leukæmia was originally obtained from Dr. Lloyd Law. Four groups of *CDBA* mice were given intraperitoneal injections of 1–5 million leukæmic cells. Three groups of 7 mice each were treated 24 hr. later with a single intraperitoneal injection of 'Cytoxan' of 150, 180, or 200 mgm./kgm. An untreated group served as a survival control. The first mouse to develop extensive ascites in the 200 mgm./kgm. treatment group was considered most likely to harbour resistant leukæmic cells. Ascites was transferred from this animal into mice for the succeeding experiment. These animals were similarly grouped and treated. This procedure was repeated serially. The median survival for untreated mice was 7–10 days. The median survivals of mice treated with 200 mgm./kgm. exceeded those of untreated mice by 88–143 per cent. Resistance was recognized in the seventh transfer generation by a decrease in median survival of treated mice to 30 per cent beyond controls. This resistant tumour was carried in the absence of drug for 12 generations, and maintained its resistance, as shown in Fig. 1. The percentage increase in median survival beyond controls for the

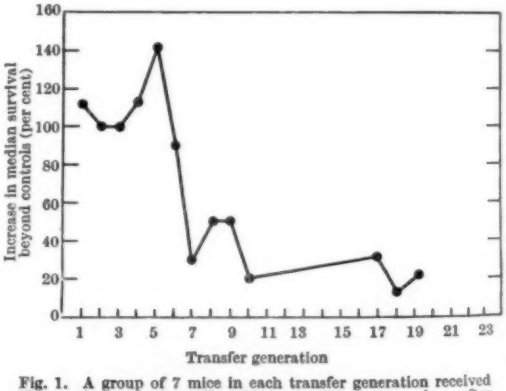

Fig. 1. A group of 7 mice in each transfer generation received 'Cytoxan' intraperitoneally, 200 mgm./kgm., one day after tumour implantation. The percentage increase in median survival beyond controls is shown. *CDBA* male mice were used

200 mgm./kgm. group is plotted for each transfer generation. Grossly and microscopically, the characteristics of this resistant variant, designated *L*-1210/*Cy-R*, and the parent leukæmia are similar, as are the courses of the disease produced by each.

Resistance was also demonstrated by the following experiments. A single intraperitoneal injection of 'Cytoxan' of 250 mgm./kgm. was given to mice 1 hr. after subcutaneous implantation of 10^5 tumour cells of the sensitive leukæmia. The drug prevented these cells from becoming established, and treated mice survived. Mice similarly treated after implantation with *L*-1210/*Cy-R* tumour cells, however, died of leukæmia. Fig. 2 shows the effects of repeated weekly intraperitoneal treatment on the survival of mice bearing 5-day implants of the sensitive and resistant leukæmias. A 275 per cent increase in survival beyond controls was obtained with mice bearing the sensitive leukæmia, compared with only 30 per cent for those with the resistant subline.

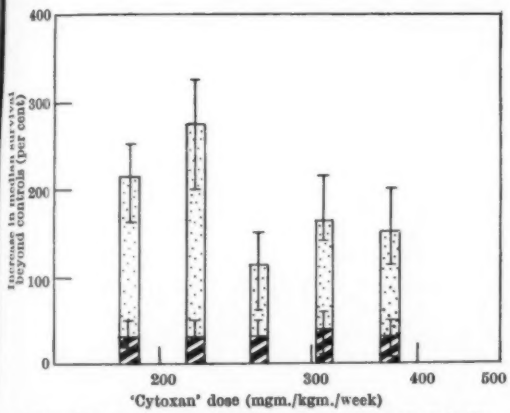

Fig. 2. The total bar measured from the abscissa represents the percentage increase in median survival beyond controls for 10 mice with the sensitive leukæmia (stippled areas), and for 10 mice with the resistant leukæmia (hatched areas). Fifteen untreated controls were used for each leukæmia. *CDBA* male mice were used. Intraperitoneal implant. Treatment: intraperitoneal weekly from the fifth day

L-1210/*Cy-R* is now in the forty-second transfer generation in the absence of drug exposure and has remained resistant. Preliminary experiments indicate that *L*-1210/*Cy-R* is sensitive to treatment with the antimetabolites, 6-mercaptopurine and 'Methotrexate', but cross-resistant to the alkylating agents uracil mustard, triethylene melamine, and triethylene thiophosphoramide.

MONTAGUE LANE*
SIDNEY T. YANCEY

General Medicine Branch,
National Cancer Institute,
Bethesda 14, Maryland.

* Present address: Department of Pharmacology (Medicine), Baylor University College of Medicine, Houston 25, Texas.

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RADIOBIOLOGY

Localization of Radioactivity in Tumours of Mice injected intravenously with Killed, Radioiodinated *Brucella abortus*

WORKERS studying the phagocytic activity of tumour cells seem to prefer *in vitro* systems[1,2]. Little work appears to have been done on the ability of tumour cells to take up colloidal or particulate substances *in vivo*. Because of the possible practical importance of the subject, it was decided to study the reticulo-endothelial activity of mouse tumours. Acetone-killed, radioiodinated *Brucella abortus* (^{131}IBr) were chosen as the test particle, since previous work[3] had shown that these bacteria are a very sensitive indicator of phagocytic activity.

Mice bearing 14–16 day old Krebs's carcinoma or 8–12 day old sarcoma-37 were used. The carcinoma was implanted into the femoral muscles of the left hind leg. The sarcoma was implanted under the skin in the region of the dorsal or lumbar vertebræ. Transplantation was performed with either trocar or syringe. In the latter case, 0·1–0·5 ml. of a dense suspension of tumour cells in phosphate buffer saline, *p*H 7·2, were injected. Details on preparation of bacteria, iodination and counting techniques, etc., have already been described[4].

Groups of 5–6 mice bearing sarcoma-37, as well as untreated controls, were given intravenously (into the tail vein) 0·1 mgm. labelled *Brucella* in 0·2 ml. saline. The mice were killed after 1 hr. and the organs removed for radioactivity determinations. Blood samples were obtained by bleeding the mice from the cut neck blood vessels, while under ether anæsthesia. The blood was collected in tubes containing citrate or heparin as anticoagulants. In several experiments, the radioactivity in tumours of mice injected with ^{131}IBr was compared with that in tumours of mice injected with radioactive iodide. In these experiments the procedure was somewhat modified: immediately before injecting the labelled material, 5 mgm. sodium iodide were administered intra-abdominally to all mice. An additional 0·8 mgm. sodium iodide was included in the intravenous inoculum. The mice were killed 5 hr. after injection. This procedure was adopted in order to reduce the uptake of labelled iodide by the tumours.

The results are summarized in Table 1; they show that in both types of experiments a significant amount of radioactivity was found in the tumours. The distribution of the label injected as iodide was completely different from that of the label injected as iodinated bacteria.

The amount of radioactivity in Krebs's carcinoma was smaller than in sarcoma. At 1 hr. after inoculation, carcinoma-bearing legs contained about 3 per cent of the injected radioactivity, as compared to 1 per cent in the normal leg.

The radioactivity in the tumours was, in general, proportional to the weight of the tissue, although this relationship did not always hold for the individual animals. Approximately 2·4 per cent of the injected radioactivity could be found in 1 gm. of tumour at 1 hr. after inoculation.

These experiments, while demonstrating the localization of radioactivity in the neoplasms, did not provide any clues as to the mechanism involved. Attempts to detect intracellular *Brucella* in tumour

Table 1. Mean Radioactivity in Organs of Mice injected with Radioiodinated *Br. abortus* or Radioactive Iodine (per cent of Injected Radioactivity)

Exp. No.	Description of mice	Material injected	Radioactivity in organs				Remarks
			Tumour	Spleen	Liver	Blood (1 ml.)	
1	Normal	Iodine-131 *Brucella*	—	9·7	23·9	9·5	Organs removed 1 hr. after injection of label
	Tumour-bearing*	Iodine-131 *Brucella*	6·1	6·1	27·7	6·9	
2	Tumour-bearing	Iodine-131 as iodide	1·1	0·06	0·5	1·0	Organs removed 5 hr. after injection of label
	Tumour-bearing	Iodine-131 *Brucella*	3·1	9·7	21·0	1·3	

* This group contained six animals. All others contained five animals. The radioactivity bound to *Brucella* was 96,000 c.p.m. That injected as iodide was 121,000 c.p.m.

smears stained with May–Grünwald–Giemsa were inconclusive, apparently because of the small size of this organism. When, however, a dense suspension of heat-killed group *A Streptococci* were injected, unmistakable proof of phagocytosis by both neoplastic and stroma cells was obtained (Fig. 1). It should be added that much less radioactivity was localized in the tumour with labelled *Streptococci* than that with labelled *Brucella*.

It appears, therefore, that phagocytosis is at least one of the mechanisms leading to concentration of radioactivity in the tumour tissue. It will be interesting to see whether these results can be reproduced in man, since they might form the basis of an additional diagnostic tool for the detection of cancer. Full details of this work will be published elsewhere.

I am grateful to Prof. J. Gross, of the Department of Experimental Medicine and Cancer Research, for discussions and suggestions during the course of this work, to Mr. G. Rasooly and Miss N. Grünwald for technical assistance, and to Dr. M. Pikovski for the supply of tumours.

D. Sulitzeanu

Department of Bacteriology,
Hebrew University–Hadassah
Medical School,
Jerusalem.

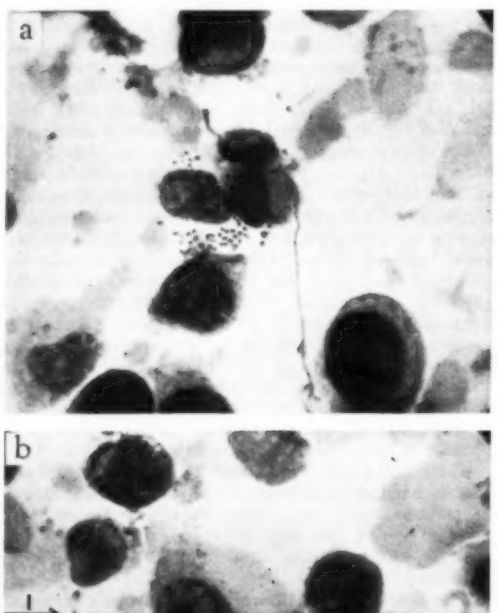

Fig. 1. *a*, *Streptococci* phagocytosed by tumour stroma cell: *b*, 1, extracellular cocci, 2, two pairs of cocci engulfed in nuclear vacuoles of malignant cell, 3, one pair of cocci in the process of being phagocytosed

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Rate of Formation of Atropine in *Atropa belladonna* Plants

We have studied the biogenesis of atropine-^{14}C in *Atropa belladonna* plants by a method which we believe to be novel in this field, but which is comparable with that applied so successfully by Calvin[1] to the elucidation of the path of carbon in photosynthesis.

A number of complete plants 2–3 months old were allowed to assimilate a single dose of carbon-14 dioxide by photosynthesis in the leaves, and were then left to continue growth under normal greenhouse conditions. At various intervals plants were killed and extracted to determine the soluble and insoluble activity remaining in the shoots and roots, and the activity in the form of atropine.

In a typical experiment, a 2–3 month old *Atropa belladonna* plant was placed in a photosynthesis chamber and fed with carbon-14 dioxide (1 mc.). It was found that more than 90 per cent of the activity was taken up by the plant during 4 hr. photosynthesis. The plant was removed from the photosynthesis chamber and grown under hydroponic conditions with the roots immersed in nutrient solution[2], in a greenhouse. After a suitable time of 'active' growth, the shoots and roots separately were continuously extracted for three days with boiling 80 per cent aqueous ethanol (about 250 ml.) and the activity of the extracts measured. Most of the solvent was removed under reduced pressure, and 0·2 *N* sulphuric acid (200 ml.) added. This acid solution was extracted with chloroform/ether (20 : 80) until the extracts were colourless, and after basifying the aqueous solution with ammonia, the alkaloids were extracted with the same solvent mixture. The alkaloid extract was dried with sodium sulphate, the solvents removed

Table 1

Plant No.	Active growth (time in days)	Soluble shoot activity (μc.)	Soluble root activity (μc.)	Total soluble activity (μc./gm. dry weight)	Insoluble shoot activity (μc.)	Insoluble root activity (μc.)	Total insoluble activity (μc./gm. dry weight)	Dry weight of plant	Ratio of atropine activity in μc./gm. dry weight to activity remaining in plant in μc.	Ratio of atropine activity in μc./gm. dry weight to soluble activity in μc.
1	0·2	465	4	260	148	1	83	1·82	$0{\cdot}071 \times 10^{-6}$	$0{\cdot}094 \times 10^{-6}$
2	4	216	39	333	201	8	270	0·77	35×10^{-6}	64×10^{-6}
3	5	*	*	91	*	*	200	2·0	39×10^{-6}	237×10^{-6}
4	6	199	61	230	301	30	290	1·15	83×10^{-6}	190×10^{-6}
5	7	190	39	153	333	43	295	1·53	21×10^{-6}	56×10^{-6}
6	10	*	*	200	*	*	350	0·81	115×10^{-6}	248×10^{-6}
7	13	*	*	140	*	*	85	2·16	21×10^{-6}	33×10^{-6}
8	16	*	*	62	*	*	110	2·17	60×10^{-6}	160×10^{-6}
9	20	94	13	80	275	67	300	1·13	136×10^{-6}	571×10^{-6}
10	21	102	2	70	247	35	230	1·24	83×10^{-6}	308×10^{-6}
11	22	44	10	50	150	41	170	1·11	120×10^{-6}	540×10^{-6}
12	23	42	5	55	94	50	160	0·86	164×10^{-6}	666×10^{-6}
13	25	*	*	90	*	*	200	1·43	118×10^{-6}	380×10^{-6}
14	30	*	*	35	*	*	90	2·67	27×10^{-6}	103×10^{-6}
15	31	61	44	50	133	92	100	2·21	220×10^{-6}	71×10^{-6}

* Not determined.

nder reduced pressure, and the alkaloids purified by hromatography on paper in butanol/water/acetic cid[3]. The atropine was washed from the paper, and arrier atropine (100 mgm.) was added and then onverted into the picrate, which was crystallized everal times from hot water and had melting point 176° C. Dilution analysis of the atropine-^{14}C picrate ndicated a radiochemical purity greater than 98 per ent.

The picrate was burnt in a stream of dry oxygen and the carbon-14 dioxide absorbed in hot barium hydroxide. The precipitated barium carbonate-^{14}C was counted in a calibrated 2π-flow counter using argon/methane (90 : 10) as the flow gas. The insoluble plant residues were ground to a fine powder and also converted into barium carbonate-^{14}C by combustion in oxygen as above. The activity of the barium carbonate in this case was measured with an end-window counter.

The results of a typical series are presented in Table 1.

The figures are erratic, and we consider that experimentation on a larger scale than we were able to undertake is called for to achieve results which will be statistically more than roughly quantitative. We wish, however, to direct particular attention to the figures in the last column, which show the atropine activity by comparison with the soluble activity, both derived solely from the single initial dose of carbon-14 dioxide. A pronounced maximum is shown at 20–25 days, and a less pronounced maximum at 5–10 days. The total soluble activity, however, reaches a maximum within a few hours and thereafter decays, roughly exponentially, to about 40 per cent at 7–10 days and 20 per cent at 21 days, of the initial value. The total insoluble activity at first increases rather slowly and then falls off.

We think that these results of a preliminary investigation are relevant to the question of whether the biogenesis of atropine (or indeed of alkaloids generally) is an 'upgrade' process from small molecules or a 'downgrade' process from larger molecules[4]. In conjunction with published results from precursor studies[5], they lead us to suggest that both processes are involved but that a 'downgrade' process plays the greater part, since the maximum atropine labelling appears so long after the activity and specific activity of the soluble carbon-14 have passed their maximum values.

These experiments arose from what was originally planned to be a series of experiments with labelled precursors to obtain evidence on the theories put forward by Robinson[6,7]. Some biosynthetic atropine-^{14}C was desired in the first place to investigate the rate of turnover in the plant, and we were surprised to find that a fairly long period was required to produce appreciable labelling from a dose of carbon-14 dioxide. Evidence on this rate of turnover is still lacking. We have, however, fed methylamine-^{14}C hydrochloride (1 mc., 3·6 mgm. in 180 ml.) via the roots of *Atropa belladonna* plants and extracted the atropine after nine days. Although the amine was readily taken up by the plant, the atropine activity (in μc./gm. dry weight/μc. of activity remaining in the plant) was only 37×10^{-6}. This comparatively low figure suggests that methylamine as such is not in fact directly incorporated in the biogenesis of atropine.

We believe that the method of a single dose of carbon-14 dioxide followed by normal growth and periodic analysis might profitably be more widely used in problems of this kind, since it offers the closest possible approach to normal physiological conditions in tracer experiments. It is susceptible of refinement in that the relative labelling in different carbon atoms might be determined after various growth periods. It is a complicating feature of atropine that the tropine and tropic acid moieties doubtless arise by different biogenetic paths and no doubt become labelled at different rates, although as yet we have not investigated this point.

We thank Dr. W. P. Grove for his interest in this work and one of us (E. A. E.) thanks the United Kingdom Atomic Energy Authority for the award of a Harwell fellowship (1955–58).

J. R. Catch
E. A. Evans

The Radiochemical Centre,
Amersham,
Buckinghamshire.

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BIOLOGY

Prolonged Survival of Human Sperm in chemically defined Media at Room Temperatures

It has been pointed out that mammalian sperm represents an ideal tissue for exploring, under well-defined[1] conditions *in vitro*, the intricate biochemical and physiological mechanisms underlying the viability and senescence of living cells[1]. Efforts to move in this direction have been restricted by the necessity of having to maintain mammalian sperm in complex, undefined media and at temperatures which suppress metabolic activity.

Our success with the maintenance of functionally and metabolically active bovine spermatozoa at room temperatures[2] prompted us to investigate the possibilities of sustaining human sperm at close to physiological temperatures in various chemically defined maintenance media. Of all the media tested, medium 199 (Morgan, Morton and Parker[3]), a product of Hyland Laboratories in California, provided human sperm with the most satisfactory and compatible environment, which was capable of supporting their survival and activity for several days at room temperatures (25 ± 2° C.).

The procedures employed were as follows: Semen collected from human donors was pooled immediately after liquefaction. The spermatozoa were washed free of seminal plasma by centrifugation (700*g* for 15 min.). After washing twice in the medium under investigation, the final volume was adjusted to give a concentration of 5–10 million cells/ml. of sterile medium. All media tested contained the following additives in final concentration: 31 mgm./100 ml. penicillin (sodium), 68 mgm./100 ml. dihydrostreptomycin sulphate, 300 mgm./100 ml. sulphanilamide, 4 units mycostatin per ml. and 150 units aqueous solution of sterile catalase (Worthington Biochemical Corporation) per ml. The suspension of human sperm was poured into small, sterile plastic vials, filled as completely as possible and stored in the dark[4] at room temperatures. Initially and daily afterwards, separate unopened vials were removed, inverted several times to provide a uniform suspension of cells and measurements taken to assess motility and viability. The concentration of live sperm was determined by means of the eosin–nigrosin differential staining technique[5]. The vigour of the progressive motility was rated by means of an arbitrary scale graduated from 0 (no motility) to 5 (excellent progressive motility). The number of motile cells was calculated by killing and counting all the dead, non-motile cells in one hæmocytometer and counting the non-motile cells in a second hæmocytometer. The difference between these two counts represented the concentration of motile sperm in the population. *p*H measurements were taken at various intervals during the experiment.

Table 1 summarizes the results of three replicate experiments. The number of live and progressively motile cells suspended in medium 199, buffered with 0·02 *M tris*, remained relatively constant for five days. It should be noted that medium 199 buffered with bicarbonate and carbon dioxide did not sustain cell viability as well as media buffered with *tris* or phosphate.

The *p*H of the *tris*-buffered 199 sperm suspension, initially 7·1, was usually lowered 0·1–0·2 of a *p*H unit on the fifth day of storage. Changing the medium on the third or fifth day of storage did not appreciably affect the time of survival of the sperm. However, the slight increase in alkalinity of the fresh medium noticeably increased the vigour of their translatory motion. The introduction of a small amount of egg yolk (0·5–1·0 per cent) into the medium significantly enhanced both the progressive motility and the survival time of these cells.

Table 1. Effect of *Tris*-Buffered Maintenance Medium 199 on Motility and Survival of Human Spermatozoa kept at Room Temperatures for Six Days

Age of sperm (days)	Live (per cent)	Motile (per cent)	Quality of motility*
0	60	67	3·5
1	58	64	4·0
2	52	51	3·5
3	55	54	3·5
4	49	55	3·5
5	45	50	3·5
6	34	37	3·0

* 0 (non-motile), 1 (vibratory), 2 (poor progressive), 3 (fair progressive), 4 (good progressive), 5 (excellent progressive).

This technique makes possible a more comprehensive investigation into the metabolic and functional behaviour of young and aged post-mitotic human cell populations, under well-defined and rigidly controlled conditions.

The work was supported by grant *RG*-6339 from the National Institutes of Health and is published with the approval of the director of the West Virginia Agricultural Experiment Station.

Charles Norman
Erwin Goldberg
I. D. Porterfield
C. E. Johnson

Departments of Biology and Dairy Science,
West Virginia University.

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Development of the Electric Organ of Mormyridae

The existence of striated myofibril structures persisting in the electroplaques of adult fish had led several authors[1–3] to consider muscle as the tissue of origin of the electric organs of Mormyridae, but no data have been published to justify this assumption. Recently I have presented circumstantial evidence showing indirectly that the electric organ of the Mormyridae does in fact arise from the deep lateral muscle[4]; however, direct experimental evidence was still lacking.

Through the courtesy of M. J. Daget, head of the Hydrobiological Laboratory of the Institut Français d'Afrique Noire, Diafarabé, Sudan, I had the opportunity of examining a batch of young Mormyridae and am now able to provide direct evidence on the origin and ontogenetic development of the electric organ of these fish.

As is well known, all species of the family Mormyridae possess two pairs of organs situated symmetrically on each side of the vertebral column between the caudal, dorsal and anal fins[5]. In the adult fish each organ is composed of a single series of electroplaques,

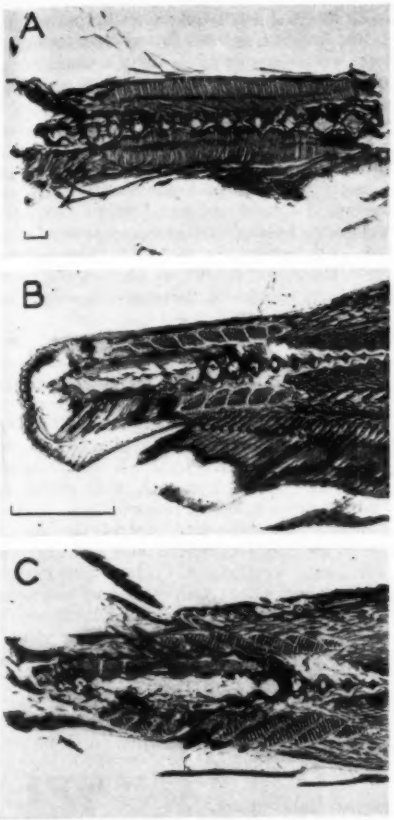

Fig. 1. Longitudinal sections of tails of three Mormyridae. *A*, *Mormyrops deliciosus* (120 mm.) adult; *B*, *Hyperopisus occ.* (24·5 mm.); *C*, *Mormyrus rume* (24·5 mm.); both young. Hæmatoxylin – eosin stain. Scale, 1 mm.

which are juxtaposed and are variable in number, 40–200 according to the species. The electroplaques are of the same size and are situated perpendicularly to the longitudinal axis of the fish. The very regular disposition of the electroplaques (Fig. 1*A*) seems to indicate that the organ arises from a special centre.

However, longitudinal histological sections of the tail of a very young *Mormyrus rume* (Figs. 1*C* and 2*A*) show that the electric organ, which is of uniform structure in the adult, is here composed of about ten groups of electroplaques. Each group is situated within a single myomere and occupies only the portion adjacent to the vertebral column. The plaques are arranged perpendicularly to the myoseptal wall in the same way as the muscle fibres. The nerve trunk is situated in the myosept and the nerve fibres penetrate only between those electroplaques which are situated in the myomere anterior to the myosept; only the caudal surfaces of the electroplaques receive nerve endings.

Although the organ is not fully developed, the majority of the electroplaques have their final aspect: double electroplasmic wall in the form of an envelope, enclosing a non-electroplasmic striated structure; the surface of the posterior wall has cylindrical prolongations (pedicles) of which only the extremity is surrounded by nerve endings.

However, in the neighbourhood of the intact fibres of the myomeres which form part of the organ, electroplaques in different evolutionary stages may be seen: for example, in one case, the electroplasm is fully formed, but does not yet possess any pedicles; the plaque is still oval and the interior is often markedly striated. In other cases, the electroplasm is only partially formed; it is rich in nuclei and surrounds the caudal part of a bundle of muscle fibres of which the cranial extremity is still attached to the myosept. The striation of these muscle fibres is clearly visible, although it is less-strongly coloured than that of the neighbouring muscle tissue.

The following preliminary conclusions can be drawn from these observations: (1) The electric organ of *Mormyrus rume* arises from several myomeres; (2) the electroplaques of these organs develop from striated muscle fibre already formed; (3) in contrast to the electroplaques of *Gymnarchus niloticus* (family Mormyridae), those of *Mormyrus rume* are formed from the muscle tissue of a single myomere; (4) the pedicle is probably formed during a later stage of the development of the electroplaque; (5) the innervation is of monoseptal origin.

We have also shown the plurimyomeric origin of electric organs in other species of Mormyridae; thus in the organ of a *Hyperopisus occidentalis* (24·5 mm. in length) (Figs. 1*B* and 2*B*) and a *Mormyrops deliciosus* (38 mm. in length), the myomeric segmentation is still evident. These two fishes were older than the *Mormyrus rume* previously described, and all their electroplaques are at the same stage of development; they still show, however, a marked difference in comparison with electroplaques of the adult fish, which in these two species present a peculiarity. In contrast to *Mormyrus rume*, in which the anastomizing pedicles are fused on to the surface of the electroplaque nearest to the pedicle, the pedicles of the adult *Hyperopisus occidentalis* and *Mormyrops deliciosus* pierce and traverse the electroplaque and fuse on to the surface which is opposite to the pedicle[6]. We also know that excitation arriving from the extreme point of the pedicle is propagated towards the electroplaque and is distributed in the first place over the surface of the electroplaque opposite to the pedicle (Szabo, Th., unpublished observations).

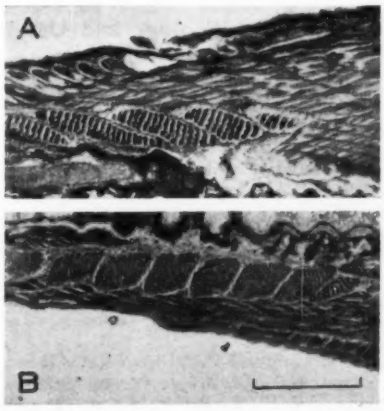

Fig. 2. *A*, Higher magnification of Fig. 1 *C*. *B*, longitudinal section of the electric organ of *Hyperopisus occ.* In both cases the myomeric segmentation is still evident. Hæmatoxylin – eosin stain. Scale 0·5 mm.

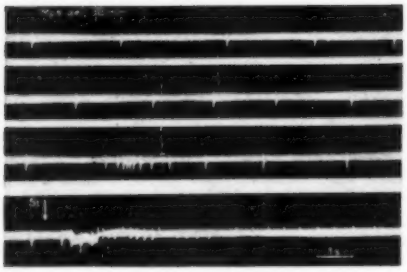

Fig. 3. Oscillographic recording of the electric activity of a *Mormyrops deliciosus* (38 mm.): electric activity of the resting animal (first three recordings) interrupted (arrow *St*) by cutaneous stimulation, provoking an acceleration of the spontaneous emission

In the very young fish, although the electroplaques already possess their long pedicles and these their nervous endings, the pedicles are attached to the surface of the electroplaque nearest to the pedicle and do not traverse the electroplaque. Accordingly, microscopic examination reveals no discontinuity in the electroplaque. Oscillographic recordings also prove that in these young electroplaques, which already function (Fig. 3), it is the surface nearest to the pedicle which is initially active and not the opposite surface, as in the adult.

In summary, there seem to exist three stages in the development of the pedicled electroplaques of Mormyridae: (1) formation of the electroplasmic tissue; (2) consecutive rapid appearance of the pedicle and its innervation; (3) piercing of the electroplaque, occurring in certain species at a later stage of development.

TH. SZABO

Centre d'Etudes de Physiologie nerveuse,
4 avenue Gordon-Bennett,
Paris, 16e.

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The Manatee: Ecology and Use for Weed Control

SINCE 1885 the Botanic Gardens in Georgetown, British Guiana, has had in some ornamental pools manatees (*Trichechus manatus*, order Sirenia), which have been always fed with grass by visitors. These pools have been kept clear by the manatees and it seemed always necessary to get additional food in the form of grass. It was therefore felt that experiments should be proceeded with using these animals for weed control, and some have been maintained in the reservoirs of the water purification plant and have effectively kept these clear of aquatic plants, particularly *Cabomba aquatica*, *Anacharis*, *Leersia*, *Utricularia*.

We have since found that the manatee is not merely an occasional but also a very voracious and determined grass feeder, which can in fact clear trenches which are covered with grass or *Nymphaea*, *Nelumbo*, *Eichornia*, as well as submerged aquatics, and they have been doing a very commendable job in confined areas. During 1959–60 several experiments were carried out, and it has been found that two manatees 7½ ft. long were capable of clearing a canal 22 ft. wide and 1,600 yd. long in 17 weeks. They will also keep it clear of weeds to allow the free passage of water and will break any wood barrier fixed across the canal in order to get at grass beyond the barrier. These animals are not numerous, they are perfectly docile, easily bruised and seemingly very slow in reproduction. I have collected 46 of them to date, and they have been distributed in several canals of drainage and irrigation schemes throughout the country. They are found in the rivers of British Guiana and are caught by fishermen in seines, but can easily be drowned.

Much information has been collected on their anatomy through the University of Florida and much more information is desired on their ecology and reproductive habits. I would like to have any further information available. I have already had inquiries from Ceylon, Thailand and Malaya as to the prospects of the use of these animals in those countries for weed control of the water hyacinth and other aquatic plants which are a costly nuisance.

As a means of water-weed control, the use of these animals is far more effective and lasting than the usual chemical weedicides. They are harmless to fishes and other water animals, and are seemingly unaffected even by electric eels which are normal inhabitants of some of the irrigation canals into which these manatees were introduced. The only risk in their wide use is that they may be slaughtered for their meat; legislation is now being enacted to ensure their complete protection.

A full account of ecological work on these mammals will shortly be published.

W. H. L. ALLSOPP

Fisheries Laboratory,
Department of Agriculture,
P.O. Box 174,
Georgetown,
British Guiana.

Equivalent Pore Radius in the Axolemma of the Giant Axon of the Squid

PREVIOUS work[1,2] on the fine structure of the giant nerve fibre of the squid has revealed the existence of slit-like channels ~60 Å. wide by ~5·3μ long traversing the Schwann cell along a tortuous path the total length of which is ~4·3μ. The axolemma itself is surrounded by a space ~83 Å. wide. It has been demonstrated that no effective osmotic pressure can be developed across the Schwann cell layer. Hence the axolemma appears to be the barrier for osmotically induced flow into the axoplasm.

The present communication deals with the determination of the reflexion coefficient σ of the axolemma for a set of non-electrolytic molecules of small radii. The σ values were then used (based on the principles developed by Durbin, Frank and Solomon[3], Durbin[4] and Solomon[5]) to calculate an equivalent pore radius for the axolemma in resting condition.

The giant nerve fibre from the first stellar nerve of the squid *Doryteuthis plei* was used. The technique and equipment were the same as those previously described[2]. The experiments were carried out at 23° C.; they consisted in measuring the volume changes of the axon, initially in artificial sea water (1,010 mOsm./l.)[6], and at various time-intervals after

this medium had been removed and replaced by a test solution. Test solutions were prepared by adding a known amount of non-electrolyte, usually 60, 230, and 480 mOsm./l., to artificial sea water made hypotonic (780 mOsm./l.) by the subtraction of 230 mOsm. of sodium chloride per litre. The cryoscopic point of a solution that has a concentration of 1 Osmol. per kgm. of water is −1·858° C. The probing solutes were methanol, formamide, ethanol, urea, ethylene glycol and glycerol. Their molecular radii were obtained from Fisher–Hirschfelder–Taylor models (Table 1).

Table 1. Reflexion Coefficient of the Axolemma for the Test Solutes

Solute	Molecular radius (Å.)	No. of experiments	σ Mean ± S.E.
Methanol	1·83	7	0·35 ± 0·03
Formamide	1·96	5	0·44 ± 0·02
Ethanol	2·13	5	0·63 ± 0·03
Urea	2·17	4	0·70 ± 0·03
Ethylene glycol	2·24	3	0·72 ± 0·03
Glycerol	2·77	4	0·96 ± 0·02

The rate of volume change per unit area may be described by the following equation :

$$dV/A\ dt = L_p\ \Delta\pi_i - \sigma L_p\ RT\Delta c_s \qquad (1)$$

in which L_p is a phenomenological coefficient[7], $\Delta\pi_i$ the osmotic pressure difference of non-penetrating solutes, R the gas constant, T the absolute temperature, Δc_s the concentration difference of the penetrating solute, and σ the reflexion coefficient[8,9]. σ measures the ability of the membrane to discriminate between the particular permeable solute used, and the solvent. It is considered that osmotic balance exists across the membrane when the rate of volume change at zero time is zero. From equation 1, it can be shown that :

$$\sigma = (\Delta\pi_i/RT)/\Delta c_s, \text{ when } dV/A\ dt = 0 \text{ at } t = 0 \qquad (2)$$

The osmolar concentration difference of the penetrating solutes which equilibrate a $\Delta\pi_i/RT$ of 230 mOsm./l. for the six different test solutes was determined by graphic interpolation, and afterwards σ by using equation 2 (Table 1). As expected, σ is found to depend on the radius of the solute molecule.

Durbin, Frank and Solomon[3] have suggested, and later Durbin[4] has demonstrated, that $\sigma = 1 - A_{sf}/A_{wf}$, where A_{sf} and A_{wf} are the effective pore areas available for filtration of the solute and solvent molecules respectively. Renkin's[10] equation 19 shows that A_{xf}, the effective pore area for the passage of a molecule x, is equal to :

$$A_{xf} = A_p\{2[1-(r_x/r_p)]^2 - [1-(r_x/r_p)]^4\}$$
$$[1-2{\cdot}104\ (r_x/r_p)+2{\cdot}09\ (r_x/r_p)^3-0{\cdot}95\ (r_x/r_p)^5] \qquad (3)$$

in which A_p is the geometrical pore area, and r_x and r_p are the radii of the molecule and the pore respectively. Dividing equation 3 for a given solute (s) of known radius (r_s) by the same equation for the water molecule (the radius for water, r_w, was taken as 1·5 Å.[11]), the ratio A_{sf}/A_{wf} may be determined for any r_p value.

Our experimental values for $(1-\sigma) \pm$ one standard error plotted as a function of the molecular radius can be fitted by a curve which corresponds to an equivalent pore radius of 4·25 Å., as is shown in Fig. 1. The uncertainty in fitting the curve is ±0·25 Å. The present result is independent of the nature of the path of the ions across the axolemma.

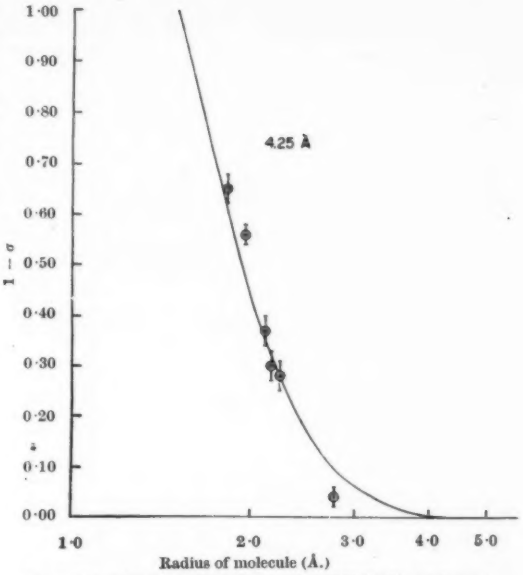

Fig. 1. Experimental values of $(1-\sigma) \pm S.E.$ for the axolemma, plotted as a function of the molecular radius. These values are fitted by a curve corresponding to an equivalent pore radius of 4·25 Å. The uncertainty in fitting the curve is ± 0·25 Å.

The presence of pores with a 4·25 Å. radius indicates that the axolemma is the membrane across which the ionic gradient necessary for nerve activity is maintained.

We are indebted to Prof. A. K. Solomon for helpful discussions during his stay at the Institute ; and to Dr. Guillermo Whittembury, Dr. Richard P. Durbin, and Dr. Leopoldo Villegas for critical comments regarding this work.

Raimundo Villegas
Flor V. Barnola

Laboratorio de Biofisica,
Instituto Venezolano de Investigaciones
Científicas (IVIC),
Caracas, Venezuela.

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Transport of Phosphorus by Fungal Mycelium

Translocation of nutrients by fungal mycelium has been reported by Schütte[1] and by Grossbard and Stranks[2]. Attempts in this Department to determine the rate of transport of phosphorus using a tracer technique have, however, indicated that the mycelium of *Phycomyces nitens*, *Absidia glauca* and *Chaetomium*

spp. is only able to transport phosphorus-32 under certain limited conditions.

The experimental procedure is to pour a synthetic agar medium plus 2 per cent glucose into Petri dishes and then to remove, aseptically, a 5-mm. wide channel along a diameter of the Petri dish. The organism is inoculated in the centre of one of the halves of the medium and grows over the channel to form a hyphal bridge between the two halves. Potassium dihydrogen phosphate, labelled with phosphorus-32, can be added to either the inoculated or the uninoculated half of the agar culture by removing a disk of agar with a cork borer and dropping the labelled solution into the well so formed. To sample, 5 mm. square portions of the agar bearing the mycelium were removed, dried on planchettes under an infra-red lamp, and counted.

The results showed that there was no translocation of phosphorus through established mycelium either from the inoculated side to the colonized side of the culture or in the reverse direction, that is, from the younger to the older part of the mycelium. The only condition under which it was possible to show transport of phosphorus in appreciable amounts was where the solution containing labelled potassium dihydrogen phosphate was added to the inoculated side of the Petri dish before growth of the mycelium over the channel had ceased. In these circumstances the mycelium takes up phosphorus which is carried by the extending hyphæ.

It seems clear, therefore, that a distinction needs to be made between translocation through established mycelium independently of the growth of the hyphæ, as in the case of movement of dyes reported in *Armillaria mellea*, *Collybia velutipes* and other agarics by Schütte[1], and transport by the uptake of material at one site and its transference in the mycelium to another site due to hyphal extension, as in the case of phosphorus reported here, and cobalt and cæsium transport referred to by Grossbard and Stranks[2]. That transport of phosphorus is via the extending mycelium itself has been confirmed in the case of *Chaetomium* sp. by transferring mycelium which had been grown on phosphorus-32 labelled medium to potato dextrose agar containing no phosphorus-32. The resultant colony consisted of mycelium which was shown both by auto-radiographs and by counting to contain phosphorus-32 up to its margin. No activity was apparent beyond the edge of the colony, indicating that phosphorus so absorbed into the mycelium does not exchange appreciably with the phosphorus present in the medium nor diffuse out into it.

R. L. Lucas

Department of Agriculture,
University of Oxford.

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GENETICS

White Band, a New Spotting Mutation in the Syrian Hamster

The first mutation reported in the Syrian hamster, *Mesocricetus auratus*, was a recessive type of white spotting which has been designated as piebald[1]. The new mutation to be described here differs from piebald in two respects: (1) the mutation is inherited as a dominant to normal pigmentation; and (2) the characteristic spotting pattern produced by the mutation is phenotypically distinguishable. The new mutant gene has been termed 'white band' and has been given the symbol *Ba*. The gene was discovered in the United States in 1957, and stock carrying the gene was imported into Britain during 1959.

Fig. 1. Three types of white spotted hamsters; the white band (left) and the piebald (right); the central animal is genetically both white band and piebald. The only interaction observed for the two forms of white is for a broadening of the medial band

With the piebald type of spotting, the white and coloured areas of the coat are greatly intermingled. There is invariably a streak of white extending from the muzzle to between the eyes. However, with the new spotting, the white areas are confined to the trunk region of the body, are not broken up to a great extent and have not been seen to occur on the head at all (see Fig. 1). The middle specimen of the photograph is a genetic combination of the two mutant genes and phenotypically displays features of both: a median band of white, which is typically wider than that of an ordinary white band, white on the forehead together with a white brindled posterior.

An imported white band male was paired with normal females, producing an F_1 of 46 white band and 43 normal offspring. An F_2 generation from the F_1 white band consisted of 90 white band and 21 normal. A backcross of the F_1 to the normal gave 79 white band and 91 normal young. A χ^2 analysis, on the assumption that the gene is assorting in exactly 50 per cent of cases from its normal allele and that the various genotypes are of normal viability, yields $\chi^2_3 = 3{\cdot}14$ ($P > 0{\cdot}30$). This value may be partitioned into a gene segregation χ^2_1 of 0·20 ($P > 0{\cdot}50$) and heterogeneity between the three generations of $\chi^2_2 = 2{\cdot}94$ ($P > 0{\cdot}20$). There is the possibility that the white band gene may be lethal when homozygous, but the frequencies of the two phenotypic classes in the F_2 are a poor fit to the 2 : 1 ratio which would be expected for this eventuality ($\chi^2_1 = 10{\cdot}82$; $P < 0{\cdot}01$).

The mutant gene derives its name from a band of white which in many individuals either completely, or almost completely, encircles the body. However, the expression of the band is variable, and in a great many animals may be partially or completely interrupted by a spinal stripe of pigment, as appears on the white band shown in Fig. 1. In high-grade animals, the band is extremely wide, the belly is almost completely white, and the areas of pigmenta-

tion are restricted to the head, shoulders and lower hindquarters.

At birth the ear auricula of most white band animals are pink, but in adult animals only the basal portions of the auricula remain depigmented. The melanism in the skin around the genitalia of both sexes is suppressed.

It is possible that the gene may not be completely dominant, since the mean percentage of white in the coat of heterozygotes, *Baba*, is less than that of homozygotes *BaBa*. The mean for the white band animals of the F_1 is $15{\cdot}87 \pm 0{\cdot}75$ per cent, for the backcross (*BC*) is $16{\cdot}34 \pm 1{\cdot}10$ per cent and for the F_2 is $23{\cdot}68 \pm 1{\cdot}3$ per cent. The F_1 and *BC* means are in good agreement, as could be anticipated, since the white bands in both generations are necessarily *Baba*. In the F_2, however, one-third of the white band animals are expected to be homozygotes and, if these possess more white than the heterozygotes, the value of the mean may be expected to be raised.

This may be the case, for the mean of the F_2 is significantly higher than the means for the other two generations. The frequency distribution of percentage of white, moreover, is unimodal and positively skewed. This would appear to indicate that, if the *Ba* gene is incompletely dominant, it is so in the stochastic sense that the higher-grade animals are more likely to be *BaBa* than *Baba*. A comparison of the frequency distribution of the F_1, *BC* and F_2 would indicate that the critical point is in the region of 35 per cent white. In the F_2, 13 per cent of the white band animals have more than this amount, whereas in neither F_1 nor *BC* did a white band approach this amount of white.

The F_1 offspring were the young of a single male, and the *BC* and F_2 generations were analysed for an association with sex. The frequencies for the *BC* are 23 ♂♂ *Ba*, 23 ♀♀ *Ba*, 17 ♂♂ *ba* and 26 ♀♀ *ba*, which yields a χ_1^2 for independent assortment of 0·84 ($P > 0{\cdot}30$). The F_2 classified for sex are 39 ♂♂ *Ba*, 51 ♀♀ *Ba*, 11 ♂♂ *ba* and 10 ♀♀ *ba*, producing an associative χ_1^2 of 0·68 ($P > 0{\cdot}03$). These results indicate that the *Ba* gene does not show partial sex linkage.

In summary, the white band trait in the hamster behaves in heredity as a dominant, monogenic, autosomal entity.

I wish to thank Mrs. Margaret Beher for her assistance in obtaining specimens of white band hamsters.

ROY ROBINSON

St. Stephens Road Nursery,
Ealing, London, W.13.

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Genetic Recombination in a Common-*B* Cross of *Schizophyllum commune*

THIS communication reports the synthesis of stable common-*B* heterokaryons in *Schizophyllum commune*, and the occurrence of normal genetic recombination in fruit-bodies derived therefrom. Although common *B* heterokaryosis and the normal segregation of the *A*-factor in common-*B* fruit-bodies have been reported in other tetrapolar species[1–3], these phenomena in *S. commune* have not been demonstrated with certainty[4,5]. The nature of production of spores in such fruit-bodies, however, has not been fully determined, and "It appears uncertain whether such fruit-bodies are apomictic or functionally bipolar"[6].

Stable common-*B* heterokaryons of *S. commune* have been established by three methods:

(1) 'Illegitimate' Buller phenomenon, for example:

$$(A^{41}B^{41}p^{r}u_1{}^{+}n_2{}^{+} + A^{2}B^{2}p^{s}u_1n_2) \times A^{2}B^{41}p^{s}u_1n_2 \rightarrow (A^{41}B^{41}p^{r}u_1{}^{+}n_2{}^{+} : A^{2}B^{41}p^{s}u_1n_2)$$

(p^s, p^r, polymyxin sensitivity and resistance, respectively; *u*, *n*, *ad*, *arg*, uracil, niacin, adenine and arginine, respectively).

(2) Isolation from the region of confluence of mated homokaryons, for example, $A^{112}B^{111} \times A^{9}B^{111}$.

(3) Nutritional selection on minimal medium from the region of confluence of mated homokaryons, for example, $A^{41}B^{42}arg_6{}^{+}ad_2n_3 \times A^{42}B^{42}arg_6ad_2{}^{+}n_3{}^{+}$. In all cases, two successive transfers of single apical cells were made to ensure the establishment of single mycelia.

The common-*B* heterokaryotic genotypes have been confirmed in all cases by mating type-tests with homokaryons, and in (1) also by the pattern of growth of the heterokaryons and of the resulting backcrossed dikaryons on various types of supplemented media, and the recovery of the individual homokaryotic component strains.

In culture, the common-*B* heterokaryon resembles a somewhat thin and irregular dikaryon and has relatively little aerial growth. The hyphæ are irregularly branched, and the septa are commonly provided with pseudoclamps, that is, incompleted clamp-connexions, the tips of which fail to fuse with the sub-terminal cells and often continue to grow to give side branches; these typically have pseudoclamps. As observed with phase microscopy, the number of nuclei per cell is 1–5. Secondary septation and the production of side branches seem to reduce the number of nuclei per cell, and these processes may account for the homokaryotic hyphæ that often grow to form distinct sectors. Preliminary results of the isolation of hyphal-tips and of the examination of mycelia grown from fragments following maceration give significant indication that the hyphæ lacking psuedoclamps are homokaryotic, whereas pseudoclamps appear to be reliable indicators of heterokaryotic hyphæ.

Common-*B* heterokaryons constituted of non-allelic, biochemically deficient strains grew normally on minimal medium: genetic complementation operates as in the dikaryon.

Small plugs of mycelium[5] taken from common-*B* matings of the type described in (3) gave growth on minimal medium only when taken from the area of confluence of the two strains: nuclear migration either does not occur at all, or it is severely restricted to a distance of no more than 2 mm.

In further analysis, two patterns of behaviour of the *B*-factor has been found.

(1) Nine independent heterokaryons of the composition ($A^{2}B^{41}p^{s}u_1n_2 : A^{41}B^{41}p^{r}u_1{}^{+}n_2{}^{+}$) were inoculated on fruiting medium in replicate sets of ten; fruiting bodies appeared in only a single culture about two months after inoculation. Small pieces of tissue isolated from the sterile portions of one of these fruits gave rise to a normal dikaryon, which by mating tests with homokaryons was found to be ($A^{2}B^{*}p^{s}u_1n_2 + A^{41}B^{41}p^{r}u_1{}^{+}n_2{}^{+}$). Among a sample of 177 monosporous progeny, there were 102 B^{41} and 75 B^{*}; a sample of 120 progeny was tested for

five additional pairs of markers (A_α, A_β, p, u n) and showed the segregation expected from regular meiosis in the diploid basidium. A test cross, $A^2B^* \times A^{41}B^2$, gave among 150 progeny 80 B^* and 70 B^2—but *no recovery* of B^{41}. All the B^* isolates of this cross had the 'flat' morphology typical of the common-A heterokaryon, and from these it proved to be impossible to recover morphologically homokaryotic mycelia, either by the isolation of single terminal cells or by deheterokaryotization by treatment with polymyxin. Together with the data on segregation, these results rule out the possibilities of disomic or heterokaryotic spores, and contamination as explanations of the 'flat' morphology. B^* is compatible with all 57 distinct B factors in the collection of these Laboratories; this includes B^{41}, only with which, however, B^* gives an anomalous reaction: irregular, patchy and delayed dikaryosis.

It therefore seems evident that these 'illegitimate' fruit bodies appeared as the result of a spontaneous mutation within the B^{41}, which led to a new B specificity and to spontaneous common-A heterokaryosis. The latter might be interpreted in two ways: (*a*) the new B factor is non-functional, therefore $A^2B^* \equiv (A^2B^1 : A^2B^2)$; (*b*) the new B is itself unstable and occasionally yields a derived factor, $A^2B^* \rightarrow (A^2B^* : A^2B^{**})$. Swiezynski and Day recently reported the "segregation of a new B reaction" in comparable fruits of *Coprinus lagopus*[3].

(2) A single, tiny fruit-body appeared on a common-B heterokaryon of the constitution ($A^{43}B^{43}arg_6ad_2{}^+n_3{}^+ : A^{41}B^{43}arg_6{}^+ad_2n_3$). This fruit-body was very similar to the aberrant fruit-bodies that sporadically appear on homokaryons. All segregants of a sample of 150 progeny that were tested, however, showed only B^{43}, but five other pairs of markers showed independent assortment and crossing-over as expected from normal meiosis.

When these results are compared with previous reports in the literature, it becomes apparent that the tetrapolar fungi, as a general phenomenon, have two kinds of hemicompatible heterokaryons: (*a*) the common-A heterokaryon is initiated by extensive nuclear migration and is characterized by depressed growth of a mycelium composed of hyphæ having simple septation[3–5]; (*b*) the common-B heterokaryon is synthesized without nuclear migration and only in the region of confluence of mated homokaryons and is characterized by the normal growth of a mycelium composed of hyphæ provided with pseudoclamps. The 'illegitimate' common-B fruit-bodies must have a normal diploid stage; therefore, they are not 'apomictic', but 'functionally bipolar'.

The results of this work will be reported in detail elsewhere.

Yair Parag
John R. Raper

Biological Laboratories,
Harvard University,
Cambridge, Mass.

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SOIL SCIENCE

A Plate Method for studying the Breakdown of Synthetic and Natural Silicates by Soil Bacteria

In a previous communication[1], a plate method for detecting phosphate-dissolving micro-organisms in soil was described. We have now found that a similar technique can be used for work on the breakdown of silicates by soil micro-organisms. For this purpose the insoluble silicates are ground in a ball mill and directly incorporated into molten agar as described for the insoluble phosphates[1].

Fig. 1 shows a number of plates prepared in this way using synthetic and naturally occurring silicates. The synthetic calcium, zinc and magnesium silicates were found to be amorphous to X-rays, but the minerals wollastonite, apophyllite and olivine gave X-ray powder diffraction patterns which showed them to be substantially free of impurities. The test organism used here was an unidentified species of *Pseudomonas* isolated from soil, and the clearing around the growth can be clearly seen.

It has already been shown by Duff and Webley[2] that 2-ketogluconic acid is produced by similar soil organisms when grown in liquid culture containing insoluble phosphates and silicates. The presence of this acid in the clearer zone around the growth in the present work was demonstrated by cutting out an agar disk (3 mm. diameter) close to the growth and applying it to a paper chromatogram and allowing it to dry at room temperature. The dried disk of extracted agar could then be brushed off and when the chromatogram was developed and sprayed, as described previously[2], 2-ketogluconic acid could be easily detected. Calcium, zinc and magnesium could usually be detected as their soluble chelates by spraying with 8-hydroxyquinoline[3].

The clearer areas containing the residues of the naturally occurring minerals (wollastonite, apophyllite, and olivine) were carefully cut out and the agar was melted in hot water. The solutions were then centrifuged to sediment the insoluble residues, which on examination were found to be amorphous by X-rays. The insoluble material from the surrounding

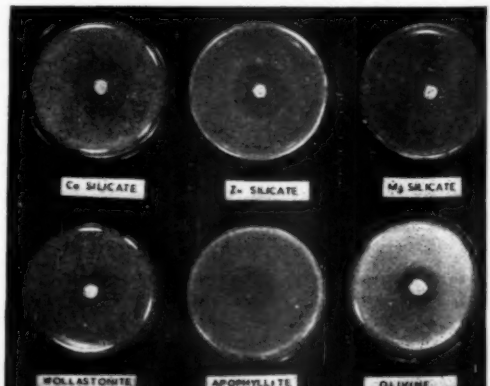

Fig. 1. Decomposition of synthetic and natural silicates by a soil bacterium. The basal medium was that used previously (ref. 1) but containing 2 per cent w/v glucose. The final concentration of the silicates was 0·25 per cent w/v. After inoculation, the plates were incubated for 4 days at 25°

cloudy zones was also extracted in the same way and gave X-ray diffraction patterns identical with those of the original powders. These results clearly showed that the crystal structure of the minerals had been destroyed.

D. M. Webley
R. B. Duff
W. A. Mitchell

Macaulay Institute for Soil Research,
Craigiebuckler,
Aberdeen.

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Some Effects of Aldrin and DDT on the Soil Fauna of Arable Land

Insecticides are now extensively applied to agricultural soils, and if this practice continues there is the obvious possibility that such treatments may have harmful effects on the soil fauna. The chlorinated hydrocarbons are not readily broken down in soil[1,2], and the general use of fertilizers containing aldrin could be one source of accumulations of this material in agricultural land. The applications of insecticide may control specific pests as desired, but could also suppress certain elements of the fauna, and even cause secondary increases of some soil animals by modifications in the predator pressure.

We have studied the effects of rates of insecticides commonly used, on the soil fauna of arable land, at Cannington Farm Institute, in Somerset. The rates of insecticides used were 3 cwt. of 1·25 per cent aldrin dust, and 2 cwt. of 5 per cent DDT dust per acre, and plots were laid out in a random block consisting of four replicates of each treatment and control. The size of each plot was 6 ft. square with 6 ft. guard rows between plots. The insecticides were applied to the surface of the plots as dusts and thoroughly mixed into the soil to a depth of 6 in. by double rotovation. All plots were kept fallow throughout the period of the trial by regular application of the weed-killers CMPP and 'Dalapon'.

The soil was sampled at intervals of 2–3 months after the beginning of the experiment. Samples taken from each plot consisted of (*a*) four 2-in. diameter cores to a depth of 6 in., and (*b*) twenty-five 1-in. diameter cores also 6 in. deep. The fauna was extracted from the first sets of samples by washing through a somewhat modified Salt and Hollick[3] apparatus and then stored in vials of alcohol until the animals could be sorted into taxonomic categories by examination under a binocular microscope. The second set of samples from each plot was bulked and thoroughly mixed, and the nematodes extracted from small subsamples placed in Baerman funnels[4].

Considerable changes in population occurred in most groups of the soil fauna. Initially numbers of most groups of animals decreased because of the cultivation, but this lasted for only three months. Both insecticides significantly decreased the numbers of Acarina in the treated plots. Aldrin also brought the population of Collembola to a low level, but DDT increased numbers of these animals to a peak nine months after treatment, after which numbers fell to a level slightly above that of the untreated plots. This confirms the results obtained by Sheals[5] for soil treated with DDT and then planted with rye grass,

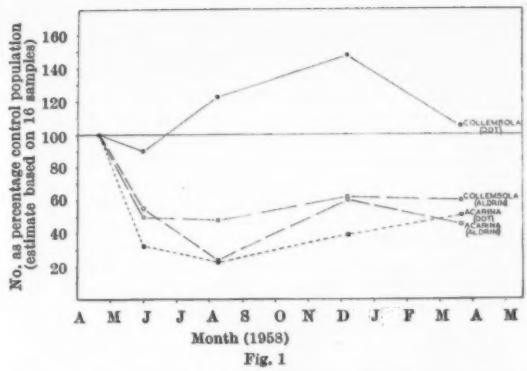

Fig. 1

and by Dobson[6], who found the Collembola population increased in arable land treated with BHC. The numbers of dipterous and coleopterous larvæ and pupæ, thrips, pauropods and symphylids were all markedly suppressed by both insecticides. Numbers of root aphids were greatly decreased by aldrin but not by DDT; neither insecticide affected the populations of small plant parasitic nematodes, earthworms and enchytraeid worms to a significant extent.

More detailed sorting of the fauna, possibly to species-level, will be necessary to show whether the increased numbers of Collembola in the DDT-treated plots result from changes in the predator–prey balance, but quite small dosages of insecticide can obviously have considerable effects on the fauna and so might eventually influence the fertility of the land.

A full account of the results will be published shortly.

C. A. Edwards
Rothamsted Experimental Station,
Harpenden, Herts.

E. B. Dennis
N.A.A.S.,
Government Buildings,
Westbury-on-Trym, Bristol.

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Microbiology of Some Soils from Antarctica

Analyses of soil from Antarctica reveal little weathering and only minute quantities of organic matter[1]. Vegetation in this area is commonly restricted to mosses, lichens, or algae. A number of samples of moss, algae and soil from the McMurdo Sound region have been examined to determine the nature of the microflora and microfauna associated with this very simple organic cycle.

Among the genera of terrestrial algae already identified are *Chlamydomonas*, *Chlorella*, *Chlorococcum*, *Stichococcus*, *Nostoc*, *Hantzschia*, *Bumilleriopsis*, *Radiosphaera*, *Heterothrix*, *Heterococcus*, all of which are typical of soils in temperate climates[2,3]. The last-named four genera have not been previously recorded from the Antarctic continent.

The bacterial flora of the moss is similar to the epiphytic flora found in temperate climates[4,5],

consisting largely of Gram-negative chromogens. The soil flora, however, appears to be more distinctive. There are more chromogenic bacteria than are normally found in soils, spore-forming bacteria are rare, and there is a large proportion of Gram-positive cocci. Attempts at isolating aerobic nitrogen-fixing bacteria were unsuccessful. Fungi are uncommon and *Streptomyces* have only been recovered from one site.

Ciliate protozoa identified include *Colpoda cucullus*, *Homalogastra setosa* and *Trichopelma sphagnetorum*, all typical soil forms[6,7], and a suctorian. Amœbæ are quite common, but testaceans have only been recovered from moss. Rotifers, tardigrades and nematodes were also present in some of the samples.

All these micro-organisms grew at room temperature, which in the case of the algae varied between 0° and 38° C. The bacteria grew both at 4° C. and at 25° C., but growth was slower at the lower temperature.

The presence of these micro-organisms, many of them typical of the soils of temperate regions, suggests the presence of an organic cycle comparable to that found in more developed soils and supports the view that the rock waste of this region may be properly referred to as soil.

Further details will be published elsewhere.

ELIZABETH A. FLINT
Department of Botany,
University of Canterbury,
Christchurch, New Zealand.
J. D. STOUT
Soil Bureau,
Department of Scientific and Industrial Research,
Wellington, New Zealand.

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VIROLOGY

Inactivation of Virus Hæmagglutinins by *para*-Chloromercuribenzoic Acid

IT has been found that a number of chemical agents reacting with thiol groups can impair the infectivity of certain viruses while leaving others unaffected[1,2].

In the course of a systematic study, one of these compounds, *para*-chloromercuribenzoic acid, has been tested for its effect on a number of virus hæmagglutinins.

It was found that substances in the media used to suspend the viruses did not abolish the effect of the compound in the concentrations used. The reagent dissolved in distilled water was mixed with equal volumes of virus suspensions and held at room temperature for 1 hr. The mixture was then dialysed at 4° against 0·1 *M* phosphate buffer, *p*H 7·2, except in the case of the arbor viruses, when borate saline, *p*H 9·0, was used[3].

The dialysing fluid was changed at 4 hr. and the dialysis continued overnight. Doubling dilutions of the dialysates were made in plastic plates, 1 per cent cells added and allowed to settle at room temperature (occasionally 4° C.). Human cells were used for most tests, sheep cells for the *E.M.C.* group, goose cells for the arbor viruses and rhesus cells for adenovirus type 7.

Table 1

Group	Virus strain and type		Log$_2$ mean reduction of titre on treatment with parachloromercuribenzoic acid 10^{-3} *M*		10^{-4} *M*	
Myxo	Influenza *A*	*W.S.*	0	(4)*	0	(1)
		Mel.	0	(1)	0	(1)
	Influenza A_2	Iksha	0	(1)	0	(1)
		A/Eng/50	0	(1)	0	(1)
	Influenza *B*	Lee	0	(3)	0	(1)
	Parainfluenza 1	Sendai	0	(4)	0	(1)
	Mumps		0	(2)	0	(1)
Adeno	Adeno type 7		>2·5	(4)	>7·5	(2)
	Adeno type 9		0	(5)	—	
	Simian virus 17		0	(3)	—	
Reo	*REO* type I		>4·4	(8)	>4	(1)
	REO type II		>1·1	(3)	>7	(1)
Entero	*ECHO* 7		>3·75	(2)	—	
	ECHO 11 prototype		>2	(1)	—	
	ECHO 11 "*U*"		2·8	(5)	>5	(1)
	ECHO 12		>1·75	(2)	—	
	Theiler's virus *GD* VII		>8	(1)	>4·5	(1)
E.M.C.	*E.M.C.*		>6	(1)	>9	(1)
	Columbia *SK*		>5	(1)	—	
Arbor	Group *A*	Semliki Forest	0	(2)	0	(1)
	Group *B*	West Nile	0	(3)	—	

* Number of tests performed. —, Not tested.

There seems to be a clear-cut division between viruses in which there is a marked destruction of hæmagglutinating activity and others which are unaffected even by high concentrations. The myxoviruses and arbor viruses seem to be unaffected while the enteroviruses and reoviruses (previously called *ECHO* 10) are inactivated. This may be a reflexion of a fundamental difference of structure between these groups. However, it can be seen that within the adenovirus group the results are not consistent and so caution would be required in using this reaction as an aid in classification. Another point of interest is that the effect of the compound on the infectivity of a virus may be quite different from that on the hæmagglutinin, for the infectivity of many myxoviruses is destroyed by this treatment whereas the infectivity of *GD* VII is unchanged.

F. E. BUCKLAND
Medical Research Council
Common Cold Research Unit,
Salisbury, Wilts.

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Intranuclear Formation of Cytomegalic Inclusion Disease Virus

HUMAN cytomegalic inclusion disease virus has been isolated in human fibroblast cultures and its cytopathogenic properties characterized by Smith[1], Rowe *et al.*[2], Weller[3], and Weller *et al.*[4]. The present communication deals with some appearances in the electron microscope. The strain used is the Kerr strain obtained from Dr. T. H. Weller which has been passaged in tissue culture about 20 times. The virus was grown in human embryonic skin-muscle fibro-

blasts[5], the medium for both growth and maintenance being Earle's saline, 0·5 per cent lactalbumin hydrolysate, 10 per cent inactivated horse serum, soya bean trypsin inhibitor and antibiotics; the *p*H was adjusted with sodium bicarbonate to 7·2. For electronmicroscopy the medium was poured off and the cells fixed *in situ* with buffered osmic acid for 1 hr. After dehydration through alcohol, the tissue was scraped off the glass and embedded in methacrylate. Thin sections were cut for examination.

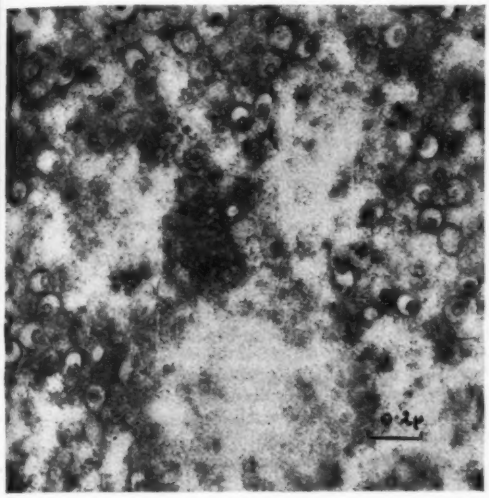

Fig. 1. Electronmicrograph showing the formation of virus particles, each possessing a single surrounding membrane, within the nucleus of the cell. (× 40,000)

Fig. 2. Electronmicrograph showing a cluster of virus particles lying close to the nuclear membrane. The latter, which is normally double, appears to be multiple over this area and shows inward extensions which are responsible for the formation around the virus particles of second and occasionally also third surrounding membranes. The large bodies in the cytoplasm are mitochondria. (× 44,800)

Fig. 3. Electronmicrograph illustrating a walled-vacuole within the nucleus, containing virus particles. Inward extensions from the inner layer of the vacuole are continuous with membranes forming the outer coats of some of the virus particles. (× 37,500)

The virus is formed in the nucleus of the cell. The earliest stage of virus formation as yet observed was that of masses of granular particles each possessing a single surrounding membrane (Fig. 1). The size of these particles is 80–120 mμ. Virus particles lying free in the cytoplasm, on the other hand, possess double membranes, the outer of which is acquired during passage through the nuclear membrane. There appeared to be two methods of passage into the cytoplasm. Many particles pass through the nuclear membrane singly (Fig. 2), and at such sites of passage there occurs an increase in the number of nuclear membrane layers, as described by Morgan *et al.*[6] for herpes simplex virus, and from which the virus acquires its outer coat. The nuclear membrane is normally double. The most striking finding, however, was the presence of many walled-vacuoles, containing clusters of virus particles, within the nucleus (Fig. 3). In many cases there was distinct evidence of continuity between the double membranes surrounding the vacuoles and nuclear membrane. They were in fact invaginations of the nuclear membrane. Both single- and double-coated virus particles were visible within them. In the case of the double-coated particles the outer coat often showed continuity with inward extensions of the inner membrane of the invagination. The presence of clusters of virus particles seems to stimulate inward movement of the

nuclear membrane resulting in the invaginations. The virus completes its formation by acquiring its outermost coat from the reduplicated nuclear membrane and the virus particles enter the cytoplasm either by rupture of the vacuole at the surface of the nucleus, or more probably by actual extrusion of the whole vacuole. Many of these virus-containing vacuoles are visible in the cytoplasm as also described by Luse and Smith[7]. The method of formation appears to resemble closely that found for herpes simplex virus in HeLa cells by Morgan *et al.*[6]

H. Stern

Dept. of Virology,
St. George's Hospital Medical School,
London, S.W.1.

I. Friedmann

Dept. of Pathology,
Royal National Throat, Nose and Ear Hospital,
London.

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Cytochemical Changes in Human Amnion Cells infected with Psittacosis Virus

Bedson *et al.*[1] described the developmental forms of psittacosis virus using conventional staining methods and light microscopy. Subsequent studies by others, who used phase, ultra-violet[2], and electron[3] microscopy, and fluorescein-tagged antibody[4], helped to describe the complex nature of the replication-cycle. In this work, we used Feulgen staining[5] and fluorescence microscopy with acridine orange dye to demonstrate cytochemical changes in tissue cultures infected with psittacosis virus. Immunocytochemical changes were followed by fluorescent-antibody techniques[6]. Pretreatment of infected cells with ribonuclease was also used in these staining procedures.

When cells stained with acridine orange are viewed with ultra-violet light, the cytoplasm and nucleoli appear rust-red and the nucleoplasm, yellow-green. In this communication, we shall refer to the rust-red colour as ribonucleic acid staining and to the yellow-green as deoxyribonucleic acid staining.

A toxigenic psittacosis virus[7] in allantoic fluid was inoculated into a line of human amnion[8] which was propagated on coverslips in Leighton tubes. At time-intervals, cultures were fixed in acid-alcohol, washed in McIlvaine's buffer (*p*H 3·8), and stained for 4 min. in 0·01 per cent acridine orange prepared in buffer. Cultures were washed and mounted in buffer. They were examined with a Reichert Zetopan microscope and illumination system. At 2–4 hr. after inoculation, deoxyribonucleic acid-staining particles (0·3–0·6μ) were noted in the cytoplasm. Within the following 24 hr., the initial deoxyribonucleic acid-staining particle was replaced by ribonucleic acid-staining inclusion which was distinct from the surrounding cytoplasm. These inclusions increased in size (1·0–4·0μ) and in numbers (2–20) until grape-like clusters were formed. During the next 48 hr., colour differentiation of the individual inclusions within these clusters occurred in sequence from shades of red to orange to yellow to green. Afterwards, and within the next 48 hr., pin-point (0·2–0·4μ) deoxyribonucleic acid-staining particles (mature virus) were evident. Re-infection occurred, and the cycle was repeated until the entire cytoplasm was filled with mature inclusions. Infected cells eventually ruptured, thus liberating virus particles into the fluid medium. Infection did not interfere with cell division; mitotic figures were noted throughout the developmental cycle.

Treatment of infected cells with ribonuclease prior to staining with acridine orange effectively removed all ribonucleic acid-staining material from the cytoplasm and nucleoli and from the matrix surrounding the deoxyribonucleic acid-staining virus particles. During all stages of development, the deoxyribonucleic acid-staining material was Feulgen positive. Preparations of the early multicoloured (red-orange) inclusions did not react with a group-related fluorescein-tagged antibody either with or without ribonuclease pretreatment. In contrast, preparations which had reached the yellow-matrix stage were antigenic, but only after prior treatment with ribonuclease. Virus which had reached the mature green-matrix and pin-point deoxyribonucleic acid-staining stages were antigenic with or without ribonuclease pretreatment. That propagation of psittacosis virus in the tissue cultures had occurred was demonstrated by intracerebral inoculations into mice.

By means of acridine orange dye and fluorescence microscopy, the replication-cycle of psittacosis virus in human amnion cells was shown to involve deposition of a ribonucleic acid-containing matrix by the host cell around an initial deoxyribonucleic acid-containing virus particle. Since this matrix could be digested with ribonuclease, it is not considered part of the intact virus and may function as a metabolic pool provided by the host cell.

This work was supported in part by the Hartz Mountain Fund and the James W. McLaughlin Fellowship Fund.

Morris Pollard
Theodore J. Starr
Richard W. Moore

Department of
Preventive Medicine and Public Health,
Virus Research Laboratory,
University of Texas Medical Branch,
Galveston.

Yoh Tanami

Department of Bacteriology, Faculty of Medicine,
Shinshu University,
Matsumoto, Japan.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 28

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. J. S. Robson: The Renal Excretion of Water in Health and Disease".*

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the London Planetarium, Marylebone Road, London, N.W.1), at 5.30 p.m.—Mr. Leonard Clarke: "Greek Astronomy and its Debt to the Babylonians".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. A. Bentz (Board of Soil Research, Hanover): "The North-west German Basin".* (First of three Special University Lectures on "The German Oil Fields and their Palæogeographic Conditions". Further lectures on November 29 and December 1.)

UNIVERSITY OF LONDON (in the Anatomy Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. John O. Corliss (University of Illinois): "Phylogeny and Evolution of the Ciliated Protozoa—Basic Concepts".* (Second of three lectures on "The Evolution of the Protozoa". Further lecture on December 5.)

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Training for Research" opened by Prof. G. H. Rawcliffe and Dr. G. A. V. Sowter.

ROYAL AERONAUTICAL SOCIETY, AGRICULTURAL AVIATION GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. J. B. Farrant: "The Farmer's Viewpoint".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the DARTFORD AND DISTRICT PHARMACEUTICAL SOCIETY, at the North-West Kent College of Technology, Miskin Road, Dartford, Kent), at 7.30 p.m.—Mr. G. L. Baldit: "Chemical Aids to Crop Protection".

Tuesday, November 29

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. L. E. Glynn: "Auto Antibodies and Diseases of Connective Tissue".* (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. A. F. Huxley: "Theories of Muscular Contraction—a Historical Survey" (Inaugural Lecture).*

ROYAL AERONAUTICAL SOCIETY, ROTOCRAFT SECTION (at 4 Hamilton Place, London, W.1), at 6 p.m.—Mr. R. Stanton Jones: "The Design of Ground Effect Machines".

IRON AND STEEL INSTITUTE (at the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 6.30 p.m.—Mr. L. Rotherham: "The Contribution of Metallurgy to Electric Power Generation" (Thirteenth Hatfield Memorial Lecture).

Tuesday, November 29—Wednesday, November 30

IRON AND STEEL INSTITUTE (at Church House, Great Smith Street, London, S.W.1)—Autumn General Meeting. Two symposia on: "Fluid Flow in Furnaces and Converters" and "Martensite".

Tuesday, November 29—Friday, December 2

CORROSION AND METAL FINISHING EXHIBITION (in the Empire Hall, Olympia, London).*

Wednesday, November 30

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Anniversary Meeting.

UNIVERSITY OF LONDON (at Bedford College, Inner Circle, Regent's Park, London, N.W.1), at 5.15 p.m.—Prof. W. F. Widdas: "Membrane Permeability and Science"* (Inaugural Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. W. J. Bray: "The Potentialities of Artificial Earth Satellites for Radiocommunication".

ROYAL AERONAUTICAL SOCIETY, MAN-POWERED AIRCRAFT GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. John Barlee: "Some Aspects of Bird Flight".

Wednesday, November 30—Friday, December 2

BRITISH PLASTICS FEDERATION (at Café Royal, Regent Street, London, W.1)—Second International Reinforced Plastics Conference.

IRON AND STEEL INSTITUTE (at the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 9.30 a.m. daily—Symposium on "Steels for Reactor Pressure Circuits".

Thursday, December 1

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (in the Empire Restaurant, Olympia, Kensington, London, W.14), at 2 p.m.—Meeting on "The Influence of Water Movement on Corrosion". Dr. G. Butler: "Ferrous Materials"; Dr. N. V. Newman: "Non-ferrous Metals".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at 10 Upper Belgrave Street, London, S.W.1), at 4.45 p.m.—Mr. J. E. Conolly: "Strength of Propellers".

INSTITUTE OF REFRIGERATION (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. E. J. Perry and Mr. P. D. Laing: "Positive Displacement Rotary Compressors as Applied to Refrigeration".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. W. R. Stevens and Mr. H. M. Ferguson: "Our Civic Lighting: Gloom or Gaiety" and "A Survey of Street Lighting and its Future".

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. Edward Ironmonger: "William Thomas Brande (1788–1866)". (Forgotten Worthies of the Royal Institution, I.)

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. B. Lennox: "Nuclear Sexing and Human Chromosomal Anomalies".* (Thirteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP and TEXTILE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Meeting on "The Microbiological Breakdown of Cellulose". Dr. J. A. Gascoigne: "The Industrial Significance of Cellulose Breakdown"; Mr. P. Russell: "Microbiological Studies in Relation to Moist Groundwood Pulp".

INSTITUTE OF METALS, LONDON LOCAL SECTION (joint meeting with the LONDON BRANCH of the INSTITUTE OF BRITISH FOUNDRYMEN (at 17 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. D. V. Atterton: "The Origin, Detection and Elimination of Gases in Cast Metals".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the BATTERSEA COLLEGE OF TECHNOLOGY CHEMICAL SOCIETY, at the Battersea College of Technology, Battersea Park Road, London, S.W.11), at 7 p.m.—Sir Christopher Ingold, F.R.S.: "Nitration and Nitrosation; a Contrast".

UNIVERSITY OF LONDON (at Wye College, Wye, Ashford, Kent), at 8.15 p.m.—Prof. A. C. Schuffelen (Wageningen): "Some Aspects of Research on Soil Fertility".*

Friday, December 2

WORSHIPFUL SOCIETY OF APOTHECARIES OF LONDON, FACULTY OF THE HISTORY OF MEDICINE AND PHARMACY (at Apothecaries' Hall, Black Friars Lane, London, E.C.4), at 5.30 p.m.—Dr. A. H. T. Robb-Smith: "The Growth of Knowledge of Functions of Blood".

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Telemetering Biological Data" opened by Dr. G. H. Byford and Dr. K. G. Williams.

BRITISH INSTITUTION OF RADIO ENGINEERS, COMPUTER GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. D. Roberts, Dr. D. S. Campbell and Mr. P. M. Thompson: "Progress in Microminiature Circuit Techniques for Digital Computers".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. W. H. Linnell: "Pharmacy—Art or Science".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. J. Z. Young, F.R.S.: "Control, Constancy and Change in Animal Life".

Saturday, December 3

BRITISH INTERPLANETARY SOCIETY (in the Tudor Room, Caxton Hall, London, S.W.1), at 6 p.m.—Dr. H. C. King: "The History and Future of Telescopes for Astronomical Observations".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER (with special qualifications in poultry husbandry) IN THE DEPARTMENT OF AGRICULTURE—The Secretary, The University, Edinburgh (December 5).

SENIOR SCIENTIFIC OFFICER (with an honours degree in physics, and several years relevant experience) AT THE DEFENCE STANDARDS LABORATORIES, Department of Supply, Maribyrnong, Victoria, Australia, to take charge of a section engaged in applying X-ray crystallography and electron microscopy to structural problems arising from the chemical, physical and metallurgical work of the D.S.L., including interpretation of results; also applying X-ray diffraction to metallurgical examinations and chemical analysis—The Senior Representative (AV.82/5), Department of Supply, Australia House, Strand, London, W.C.2 (December 7).

ASSISTANT LECTURER IN PLANT PATHOLOGY IN THE DEPARTMENT OF AGRICULTURAL SCIENCES, School of Agriculture, Sutton Bonington—The Registrar, The University, Nottingham (December 9).

ASSISTANT LECTURER or LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Sheffield (December 10).

SENIOR PHARMACOLOGIST (well versed in standard classical pharmacology); and a SENIOR PHYSIOLOGIST (well versed in mammalian experimental techniques), to take charge of sections of medical research at the Chemical Defence Experimental Establishment (War Office), Porton, Wilts—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting G.397/0A (December 10).

CHAIR OF FLUID MECHANICS WITHIN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, The University, Leeds 2 (December 14).

UNIVERSITY DEMONSTRATOR IN ORGANIC CHEMISTRY—Dr. F. B. Kipping, University Chemical Laboratory, Lensfield Road, Cambridge (December 14).

LECTURER/SENIOR LECTURER IN PSYCHOLOGY at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, December 15).

TEMPORARY RESEARCH ASSOCIATE or RESEARCH FELLOW (suitably qualified medical or science graduate with good experience in enzymology) IN THE BIOCHEMICAL SECTION OF THE DEPARTMENT OF PATHOLOGY, for work in connexion with possible enzyme disturbances in cancer—The Registrar, King's College (University of Durham), Newcastle upon Tyne (December 18).

LECTURER IN ELECTRICAL ENGINEERING—The Registrar, Manchester College of Science and Technology, Manchester 1 (December 19).

CHAIR OF PHYSICS; and CHAIR OF ZOOLOGY in the University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 30).

LECTURER IN BACTERIOLOGY at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 31).

LECTURER or ASSISTANT LECTURER IN PHARMACEUTICS at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 31).

SENIOR LECTURER IN CHARGE OF THE DEPARTMENT OF PHILOSOPHY, The University College of Sierra Leone (Fourah Bay College)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 31).

ANALYTICAL CHEMIST (with a first- or upper second-class honours degree in inorganic chemistry, or equivalent qualification, and a thorough grounding in this subject, with special reference to inorganic quantitative analysis) in Bechuanaland, to take charge of the Geological Survey Laboratory, and undertake the analysis of rocks, ores, minerals and ground-water, etc.—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.105/76/05.

ASSISTANT EXPERIMENTAL OFFICERS or EXPERIMENTAL OFFICERS (2) (with a degree in mathematics or statistics, or a degree in agriculture or biology with an interest in experimental work and statistics) IN THE STATISTICS DEPARTMENT, for work on agricultural and biological statistics—The Secretary, Rothamsted Experimental Station, Harpenden, Herts.

CHEMIST (with a good honours degree in chemistry, and at least two years postgraduate research experience, preferably in insecticide analysis), with the PESTICIDES RESEARCH UNIT, Tanganyika, for research into the chemical aspects of the control of tropical pests and to take charge of the chemical laboratory—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.87/8/01.

COMPUTER (with a competent knowledge of, and preferably a degree in, mathematics) IN THE DEPARTMENT OF PHYSICS, to assist research groups by computations using desk calculating machines and an electronic computer—The Professor of Physics, King's College (University of Durham), Newcastle upon Tyne 1.

LECTURERS, ASSISTANT PROFESSORS and ASSOCIATE PROFESSORS IN THE DEPARTMENT OF MATHEMATICS (either at the Edmonton branch or the Calgary branch of the University)—Professor E. S. Keeping, Mathematics Department, University of Alberta, Edmonton, Alberta, Canada.

TECHNICAL EDITOR (preferably with a pass degree or H.N.C., and experience in a related field of work), to be employed on editing, proof-reading, indexing and distribution of scientific reports, technical specifications, etc., and to handle related information inquiries—The Senior Recruitment Officer, Atomic Weapons Research Establishment, Aldermaston, Berks, quoting Ref. No. B.2493/34.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Agriculture, Fisheries and Food. Bulletin No. 146: Poultry Breeding: Genetics and Systems of Breeding. By G. E. Mann. Third edition. Pp. vi+146. (London: H.M. Stationery Office, 1960.) 7*s*. net. [188

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